Hong Kong -Taiwan Physiology Symposium 2012

14th June, 2012

AND

Joint Scientific Meeting of Hong Kong Society of Neurosciences & The Biophysical Society of Hong Kong

14th-15th June, 2012

ChiaYi U NCKU KMU HKU CUHK HKUST

BPHK HKSN

CONTENT

Welcome Message

Hong Kong-Taiwan Physiology Symposium

The Organising Committee is delighted to organize this year's Hong-Kong Taiwan Physiology Symposium, which is held in the School of Biomedical Sciences, The Chinese University of Hong Kong. The joint Physiology Symposium of Hong Kong and Taiwan has been run for many years and serves as a major platform for the academic exchange among scientists across the Strait in the field of physiology and other life sciences. This year, the hosts in The Chinese University of Hong Kong and the University of Hong Kong have the pleasure to welcome more than 30 faculties, research fellows and postgraduate students from Taiwan. In addition to the many excited talks delivered by faculties, to honor the pivotal contribution of Prof. Michael Tam in promoting the exchange in research and teaching between Hong Kong and Taiwan in past decades, a special symposium paying tribute to him is arranged in this meeting. This Symposim is also held back-to-back, and has a shared session, with the Joint Meeting of the Hong Kong Society of Neurosciences and the Hong Kong Biophysical Society. The merging of the two meetings provides a cherished opportunity for scientisists from the three disciplines to meet, exchange research ideas and form networks. The organization of the Symposium relies on many helping hands. We appreciate and thank all those who contribute to the realization of this Symposium. Special thanks go to the School of Biomedical Sciences of CUHK for accommodating this event in the LKS Integrated Biomedical Sciences Building and the support provided. To all participants, we hope you will enjoy this meeting!

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The Symposium is supported by

The Chinese University of Hong Kong, School of Biomedical Sciences

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Welcome Message

Joint Meeting of HK Society of Neurosciences & Biophysical Society of HK

It has been two years since our two societies, the Hong Kong Society of Neurosciences (HKSN) and the Biophysical Society of Hong Kong (BPHK), held our joint meeting. It is indeed a pleasure to welcome members of both societies and our distinguished guests to come to the Chinese University of Hong Kong (CUHK) for another exciting joint conference. We have the added pleasure this year of holding this meeting back-to-back with the Hong Kong-Taiwan Physiology Symposium 2012. The three disciplines represented by this conference, physiology, neuroscience, and biophysics, have always been intertwined historically. By bringing investigators in all three areas together, this is a forum for scientific presentation, learning and networking. This year represents a significant landmark for higher education in Hong Kong as local universities will enroll the first cohort of students into their four-year curricula. New buildings are springing up at unprecedented pace across university campuses in the territory. This brand-new, ultramodern research building at CUHK testifies to this wave of growth in Hong Kong's university system. Along with this expansion comes the influx of new scientific talents to staff our classrooms and laboratories. We hope that HKSN and BPHK will continue to serve newly arrived and existing scientists in these two disciplines to promote collegiality and exchange. We are particularly honored to have several outstanding scientists from China, Taiwan and US to deliver plenary lectures during this meeting. Equally exciting are talks to be given by a cadre of distinguished local investigators. We would also like to highlight the Young Investigators Symposiums and the poster sessions featuring local and overseas junior researchers. Adding to the excitement is the participation of students from several Asian countries attending the International Brain Research Organization (IBRO) Advanced School of Neuroscience. It is our hope that this conference will nourish old friendship, enable new relationship, catalyze scientific collaboration and promote scientific welfare of Hong Kong and beyond. To all attendees, we appreciate very much your contribution and bid you a warm welcome!

Conference Chairpersons

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Wing-Ho Yung
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Program

14 June 2012 - Morning Session

(Hong Kong-Taiwan Physiology Symposium)

08:00 - **Registration** (Foyer, Lo Kwee Seong Integrated Biomedical Sciences Building, The Chinese University of Hong Kong)

08:30 – 08:45 **Opening Address** (Room G02)
Prof. Wai Yee Chan, *Director, School of Biomedical Sciences, CUHK*
Prof. Michael SC Tam, *Associate Director, School of Biomedical Sciences, CUHK*

08:45 – 09:30 **Plenary Lecture** (Room G02)
Chairperson: Prof. Ying-Shing Chan

(PL1) UNDERSTANDING BAROREFLEX: A 30-YEAR JOURNEY — **Samuel HH Chan** *(Koahsiung)*

09:30 – 10:20 **Invited Talks** (Room G02)
Chairperson: Prof. Paul Poon

09:30 **(T1)** FLOW-INDUCED CA^{2+} RESPONSE IN CARDIOVASCULAR AND RENAL SYSTEM — **Xiaoqiang Yao** *(Hong Kong)*

09:55 **(T2)** THE INTERPRETATION OF CHI-GONG (QI-GONG) IN PHYSIOLOGIC ASPECT — **Mei-Ling Ho** *(Kaohsiung)*

10:20 – 11:00 **Tea Break &**
Poster session (P1-P26) (Foyer & Room G01)

11:00 – 12:45 **Special Symposium: A Tribute to Prof. Michael SC Tam** (Room G02)
Chairpersons: Prof. Nelson Tang & Prof. Wing-Hung Ko

11:05 **(T3)** IMPROVING THE PHARMACOLOGICAL PROPERTIES OF TRICHOSANTHIN BY SITE-DIRECTED PEGYLATION AND DEXTRAN COUPLING — **Pang Chui Shaw** *(Hong Kong)*

11:30	**(T4)** VALPROIC ACID, AN HDAC INHIBITOR, DIFFERENTIALLY INDUCES AUTOPHAGY AND CELL CYCLE ARREST IN HUMAN PROSTATE CANCER CELLS THROUGH DISTINCT EXPRESSION PROFILES OF AUTOPHAGY- AND CELL CYCLE-RELATED GENES	**Xian Hui He** *(Guangzhou)*
11:55	**(T5)** PHYSIOLOGIC GENOMICS OR GENOMIC PHYSIOLOGY: THE CHALLENGE AND OPPORTUNITY IN THE GENOMIC ERA.	**Nelson Tang** *(Hong Kong)*
12:20	**(T6)** P2Y RECEPTORS IN HUMAN AIRWAY EPITHELIA: FROM ION TRANSPORT TO AIRWAY INFLAMMATION	**Wing-Hung Ko** *(Hong Kong)*

12:45 – 14:00 **Lunch and Poster Viewing** (Foyer & Room G01)

14 June 2012 - Afternoon Session

(Shared session between the HK-Taiwan Physiology Symposium and Joint Scientific Meeting of Hong Kong Society of Neuroscience and Biophysical Society of Hong Kong)

14:00 – 14:15 **Opening Remark of HKSN-BPHK Meeting** (Room G02)
Prof. Benjamin Peng, *President, The Biophysical Society of Hong Kong*
Prof. Wing-Ho Yung, *President, Hong Kong Society of Neurosciences*

14:15 – 15:00 **Plenary Lecture** (Room G02)
Chairperson: Prof. Pingbo Huang

(PL2) MECHANOSENSITIVE ION CHANNELS: PHYSIOLOGY, PATHOLOGY, AND MOLECULAR MECHANISM — **Ching Kung** *(Madison)*

15:00 – 15:50 **Invited Talks** (Room G02)
Chairperson: Prof. Ya Ke

15:00 **(T7)** CALCIUM DYSREGULATIONS AND AUTOPHAGY IMPAIRMENT IN FAMILIAL ALZHEIMER'S DISEASE — **King-Ho Cheung** *(Hong Kong)*

15:25 **(T8)** THE ROLE OF MIR-196A IN CELL AND TRANSGENIC MOUSE MODELS OF HUNTINGTON'S DISEASE — **Shang-Hsun Yang** *(Tainan)*

15:50 – 16:20 **Tea Break &**
Poster session (P27-P52) (Foyer & Room G01)

16:20 – 18:00 **Invited Talks** (Room G02)
Chairpersons: Prof. Man-Lung Fung & Prof. Alice Chang

16:20 **(T9)** RADIATION-INDUCED INCREASE IN CELL MIGRATION AND METASTATIC POTENTIAL OF CERVICAL CANCER CELLS OPERATES VIA THE K-RAS PATHWAY — **Pei-Chin Chuang** *(Kaohsiung)*

16:45 **(T10)** UROCORTIN REDUCES CEREBRAL HEMORRAGE INJURY PARTIALLY VIA PI3K/AKT AND GSK-3B SIGNALING PATHWAYS — **Jia-Yi Wang** *(Taipei)*

17:10 **(T11)** BURSTING ACTIVITY OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR REVEALS INTRINSIC GATING SCHEME — **Jeng-Haur Chen** *(Hong Kong)*

17:35	**(T12)** DEFUNCT BAROREFLEX RESPONSES UNDERLIE CARDIOVASCULAR COLLAPSE ASSOCIATED WITH METHAMPHETAMINE INTOXICATION	**Alice Chang** *(Kaohsiung)*

19:00 **Conference Dinner, HK-Taiwan Physiology Symposium**
Royal Park Hotel (by invitation)

15 June 2012 - Morning Session

(Joint Scientific Meeting of The Hong Kong Society of Neurosciences and The Biophysical Society of Hong Kong)

08:00 - **Registration** (Foyer, Lo Kwee Seong Integrated Biomedical Sciences Building, The Chinese University of Hong Kong)

8:45 – 9:30 **Plenary Lecture** (Room G02)
Chairperson: Prof. Wing-Ho Yung

	(PL3) ALPHA-2A ADRENERGIC REGULATION OF PREFRONTAL CORTICAL FUNCTIONS	**Bao-Ming Li** *(Nanchang)*

9:30 – 10:30 **Young Investigators Symposium I** (Room G02)
Chairpersons: Prof. Kenny Chung & Prof. Daisy Shum

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9:45	**(Y2)** EXPRESSION OF TOLL-LIKE RECEPTOR 4 SIGNALLING COMPONENTS IN ADULT RAT DORSAL ROOT GANGLION CELLS	**Kai-Hei Tse** *(CUHK)*
10:00	**(Y3)** PROTECTIVE EFFECTS OF LYCIUM BARBARUM POLYSACCHARIDES ON CEREBRAL EDEMA AND BLOOD-BRAIN BARRIER DISRUPTION AFTER ISCHEMIC STROKE	**Di Yang** *(HKU)*
10:15	**(Y4)** GENOME-WIDE EXPRESSION ANALYSIS OF MIGRATORY SACRAL NEURAL CREST CELLS FROM THE DOMINANT MEGACOLON MOUSE MUTANT	**Yonghui Hou** *(CUHK)*

10:30 – 11:00 **Tea Break &Poster session (P53-P78)** (Foyer & Room G01)

11:00 – 12:45 **Hong Kong-Guangdong Biophysics Symposium** (Room G02)
Chairpersons: Prof. Guang Zhu & Prof. Jufang He

11:00	**(T13)** AUTONOMOUS FORMATION OF SEQUENTIAL PERIODIC STRIPES FROM DENSITY-DEPENDENT MOTILITY	**Jiandong Huang** *(Hong Kong)*
11:25	**(T14)** CHOLECYSTOKININ: THE MEMORY-WRITING CHEMICAL IN THE BRAIN	**JuFang He** *(Hong Kong)*
11:50	**(T15)** STRUCTURE-FUNCTIONAL STUDY OF PROTEINS THAT REGULATE CELL PROLIFERATION AND DIFFERENTIATION	**Guang Zhu** *(HongKong)*
12:15	**(T16)** BIOPHYSICAL PROPERTY OF HUMAN RED BLOOD CELL AS A FUNCTION OF CELL AGING AND PATHOLOGICAL CONDITIONS	**Yao-Xiong Huang** *(Guangzhou)*

12:45 – 14:00 **Lunch and Poster Viewing** (Foyer & Room G01)

15 June 2012 - Afternoon Session

(Joint Scientific Meeting of The Hong Kong Society of Neurosciences and The Biophysical Society of Hong Kong)

14:00 – 14:45 **Plenary Lecture** (Room G02)
Chairperson: Prof. Benjamin Peng

	(PL4) CELLULAR AND MOLECULAR MECHANISMS GOVERNING PERIPHERAL NERVE REGENERATION IN ZEBRAFISH	**Michael Granato** *(Philadelphia)*

14:45 – 15:45 **Young Investigators Symposium II** (Room G02)
Chairpersons: Prof. Amy Lo & Prof. John Rudd

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15:00	**(Y6)** EFFECT OF A MUSCARINIC RECEPTOR AGONIST AND A NITRIC OXIDE SYNTHASE INHIBITOR ON THE REGULATION OF GASTRIC MYOELECTRICAL ACTIVITY IN ICR MICE	**Eileen HC Wang** *(CUHK)*
15:15	**(Y7)** BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) REGULATES THE ACQUISITION OF VESTIBULAR-MEDIATED SPATIAL ORIENTATION IN RATS	**Francisco Botelho** *(HKU)*
15:30	**(Y8)** NANO-STRUCTURED PLATFORM FOR HIGHLY SELECTIVE, SENSITIVE AND QUANTITATIVE ELECTROCHEMICAL DETECTION OF HYDROGEN PEROXIDE (H_2O_2)AND GLUCOSE IN BIO-MIMETIC MEDIA	**Tsz Lun Wong** *(HKUST)*

15:45 – 16:15 **Tea Break & Poster session (P79-P104)** (Foyer & Room G01)

16:15 – 18:00 **Invited Talks** (Room G02)
Chairpersons: Prof. Helen Wise & Prof. Ken Yung

16:15	**(T17)** MODELING FM RESPONSES OF MIDBRAIN AUDITORY NEURONS WITH COMPLEX RECEPTIVE FIELDS	**Paul WF Poon** *(Tainan)*

16:40	**(T18)** MOLECULAR MECHANISM OF CIRCUIT FORMATION IN THE RODENT SOMATOSENSORY BARREL CORTEX	**Tomomi Shimogori** *(Wako)*
17:15	**(T19)** HELPING ISOLATED NEURONS MAKE APPROPRIATE CONNECTIONS	**Gordon Aubuthnott** *(Okinawa)*
17:30	**(T20)** HIPPOCAMPAL NEUROGENESIS FOLLOWING TRAUMATIC BRAIN INJURY	**Yi-Ling Yang** *(Chiayi)*
18:00 – 18:10	**Closing remark** Prof. Benjamin Peng	
19:00	**Conference Dinner, HKSN-BPHK Meeting** Honorary Family Restaurant, Plaza Ascot	

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Invited Talks

Young Investigators Symposium

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Plenary Lectures

Plenary lecture 1: 08:45- 09:30, 14th June

PL1

UNDERSTANDING BAROREFLEX: A 30-YEAR JOURNEY

Samuel H.H. Chan

Kaohsiung Chang Gung Memorial Hospital, Kaohsiung, Taiwan

The most fundamental mechanism in central cardiovascular regulation exists in the form of baroreflex, which provides a rapid negative feedback mechanism that dampens fluctuations in blood pressure (BP) and heart rate (HR) induced by environmental insults. The nucleus tractus solitarii (NTS) receives primary afferent information from the baroreceptors. Outputs from NTS mediate reflex control of the heart by modulating the activity of parasympathetic premotor neurons in the nucleus ambiguus (NA). Outputs from NTS also mediate reflex adjustment of sympathetic outflow to the blood vessels by modulating the activity of sympathetic premotor neurons in the rostral ventrolateral medulla (RVLM).

For more than thirty years, our group has been engaged in the mechanistic delineation of the physiological and pathological roles of baroreflex in cardiovascular regulation. During this period, we have had the good fortunate of a multitude of methodological advancements that are available for biomedical research. For example, radiotelemetry coupled with auto- and cross-spectral analysis of BP and HR allows us to differentiate baroreflex-mediated control of the heart or blood vessels in conscious animals, and animal magnetic resonance and diffusion tensor imaging in the medulla oblongata reveals whether a disruption of the functional connectivity between NTS and NA or RVLM underpins an impairment of baroreflex. Not to mention the large spectrum of biochemical and genetic tools that opens new grounds in our search for the cellular and molecular basis of baroreflex functions and malfunctions.

This presentation shall be a summary of our partnership with those methodological advancements during our 30-year journey in deciphering the intricate of baroreflex. Highlights from our work on NTS, NA and RVLM will be used to illustrate the cellular and molecular mechanisms that underpin baroreflex dysfunction in neurogenic hypertension, brain death and temporal lobe status epilepticus. My intended take-home message is that a great deal of information can be obtained from a small research theme such as baroreflex, which provides in many cases novel physiological and pathological insights.

PL2

MECHANOSENSITIVE ION CHANNELS: PHYSIOLOGY, PATHOLOGY, AND MOLECULAR MECHANISM

Ching Kung

Mol. Biol. & Dept. Genetics, Univ. Wisconsin-Madison, USA

The GPCR*-based vision, smell, and taste are thoroughly understood. However, the molecules that receive mechanical forces in hearing, touch, balance, and the forces of blood pressure and systemic osmolarity are unknown or unclear, but are likely force-sensing ion channels. Those from bacteria have been cloned and their crystal structure resolved. Such channels can be purified and reconstituted into lipid bilayers and retain their function, indicating that the gating force comes from the lipids. No animal force-sensing channels are understood at this depth.

Several families of channels are shown to sense forces in the worm, the fly, or in cultured cells. One is the TRP** superfamily, members of which appear to participate in a variety of mechanical senses. *E.g.*, TRPV4 appears to measure weight loaded on bones. Human mutations in TRPV4 cause bone-development problems resulting in symptoms from dwarfism to fetal death. Genetic experiments with TRPY1, the TRP native to yeast, implicate that the S5-S6 permeation core (and not the four S1-S4 peripheral domains) receives the force, likely from lipids.

Understanding how animals sense mechanical forces is the last frontier in sensory physiology. Its significance and challenges will be discussed.

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* GPCR: G-protein coupled receptor; ** TRP: Transient-Receptor-Potential

PL3

ALPHA-2A ADRENERGIC REGULATION OF PREFRONTAL CORTICAL FUNCTIONS

Bao-ming Li [1] and Amy Arnsten [2]

[1] Institute of Life Science, Nanchang University, Nanchang 330031, China; [2] Department of Neurobiology, Yale Medical School, New Haven, CT 06510, USA

The prefrontal cortex guides behaviors and thoughts using representational knowledge (i.e., working memory). Such cognitive abilities subserve the so-called executive functions: the ability to inhibit inappropriate behaviors, regulate selective attention, monitor behavioral actions and plan for the future. These prefrontal cortical functions are weaker in patients with attention-deficit and hyperactivity disorder (ADHD). Studies in rodents and monkeys indicate that the prefrontal cortex is very sensitive to its neurochemical environment. It has been well documented that even small changes in catecholamine produce profound effects on the prefrontal cortical functions. An optimal level of norepinephrine acting at postsynaptic alpha-2A adrenoceptors is essential for the prefrontal cortical functions. For example, pharmacological blockade of alpha-2A adrenoceptors in the prefrontal cortex markedly impairs prefrontal cortical functions and mimics most of the symptoms seen in ADHD patients, including poor working memory, poor concentration and distractibility, impulsivity and locomotor hyperactivity. Conversely, stimulation of alpha-2A adrenoceptors in the prefrontal cortex promotes the maturation of dendritic spines in pyramidal cells, strengthens prefrontal cortical regulation of behavior, and reduces distractibility. Up to date, most effective treatments for ADHD facilitate catecholamine synaptic transmission, and well likely have their therapeutic effects by optimizing the actions of catecholamine (such as dopamine and norepinephrine) in the prefrontal cortex.

Plenary lecture 4: 14:00- 14:45, 15th June

PL4

CELLULAR AND MOLECULAR MECHANISMS GOVERNING PERIPHERAL NERVE REGENERATION IN ZEBRAFISH

Michael Granato

University of Pennsylvania, Perelman School of Medicine, Philadelphia PA, USA
granatom@mail.med.upenn.edu

Peripheral nerves have retained their regenerative capacity, yet the frequent lack of functional recovery, despite meticulous surgical repair, remains an important clinical problem. Despite its clinical importance, the cellular and molecular mechanisms underlying axonal regeneration *in vivo*, are not well understood. This is in part because the cellular and subcellular events taking place during axonal regeneration- within individual axons but also in neighboring cells such as glia and immune cells– have yet to be defined. One major limitation is to monitor degenerating axons wired in their natural context in intact animals, in real time. As a first step towards answering this question, we have established a zebrafish model in which to define the interplay between injured peripheral nerves and neighboring cells, i.e. muscle, glia cells and macrophages *in vivo* (1). We will discuss ongoing efforts to characterize the cellular inactions and the molecular-genetic mechanisms governing the re-establishment of a functional motor system after nerve injury.

(1) Rosenberg, A., Wolman, M. Franzini-Armstrong, C. and Granato, M. 2012. *J. Neuroscience*, (32), 3898-3909.

Invited Talks

Session I: 09:30-10:20, 11:00-12:45, 14th June

T1

FLOW-INDUCED CA^{2+} RESPONSE IN CARDIOVASCULAR AND RENAL SYSTEM

Yao XQ
School of Biomedical Sciences, the Chinese University of Hong Kong, Hong Kong, China

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). Tetrameric assembly usually occurs between members from the same subfamily. In the present study, with the use of co-immunoprecipitation, subcellular co-localization and fluorescence resonance energy transfer (FRET), we found that TRPV4 can associate with TRPP2 and TRPC1 to form heteromeric TRPV4-P2 and TRPV4-C1 channels, respectively. In function study, both TRPV4-C1 and TRPV4-P2 are capable of mediating flow-induced Ca^{2+} influx when they are expressed in HEK293 cells. With the use of pore-dead channel mutants and pore-blocking antibody, we found that TRPV4-P2 is responsible for flow-induced Ca^{2+} entry in M1 renal cortical collecting duct cells (M1-CCD cells), whereas TRPV4-C1 is responsible for flow-induced Ca^{2+} entry in the primary cultured rat small mesenteric artery endothelial cells. Furthermore, we found that flow-induced Ca^{2+} entry in renal CCD cells and vascular endothelial cells can both be inhibited by nitric oxide, cGMP and protein kinase G. Protein kinase G phosphorylation sites were found to be located on TRPP2 and TRPC1 respectively. In summary, we found that TRPV4-P2 mediates flow-induced Ca^{2+} influx in renal CCD epithelial cells, while TRPV4-C1 is responsible for flow-induced Ca^{2+} entry in vascular endothelial cells. Protein kinase G can act on TRPP2 and TRPC1 to inhibit the flow-induced Ca^{2+} entry in both tissues.

T2

THE INTERPRETATION OF CHI-GONG (QI-GONG) IN PHYSIOLOGIC ASPECT

Mei-Ling Ho
Kaohsiung Medical University

Chi-Gong is known as a traditional Chinese way to keep healthy because it generates Chi, the mystical energy. The extremely powerful Chi is also able to be generated when practicing the Internal Fist (內家拳). Internal Fist is to fight using Chi instead of muscle power, which is the main difference from the External Fist (外家拳). Different Internal fists have been created in ancient China at different dates and areas. Shaking Crane (震身鶴) is one of the most powerful Internal fist in south China. To generate the powerful Chi, a special way that acts in coordination with breathes (內功呼吸法) is used for this purpose. In this talk, the complicated mechanical way to generate the power Chi will be present. The Chi-generation mechanism will be interpreted in the physiologic aspect including respiratory and circulatory physiology. The powerful force of Chi from Shaking Crane will be presented by video. The possible physiological mechanism for the beneficial effect of Chi on health will be suggested. The possible ways to further study the Chi effects will be discussed.

T3

IMPROVING THE PHARMACOLOGICAL PROPERTIES OF TRICHOSANTHIN BY SITE-DIRECTED PEGYLATION AND DEXTRAN COUPLING

Pang-Chui Shaw
Biochemistry Programme, School of Life Sciences, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong, China

Trichosanthin (TCS), a type I ribosome-inactivating protein (RIP), has abortifacient, anti-tumor and anti-HIV activities. In order to reduce its antigenicity and prolong its plasma half-life, several amino acids

on the surface of TCS were identified and mutated to cysteine through which PEG or dextran was covalently attached. In general, the *in vitro* ribosome-inactivating and cytotoxic activities of PEGyated TCS variants were lower than the wild-type protein. However, the *in vivo* abortifacient activity was only slightly decreased, unchanged, or even enhanced in some preparations. On the other hand, the plasma half-life of the variants was found to be increased by 6 to 50 folds in comparison to the native TCS. IgG and IgE responses of the variants were also reduced. Particularly, TCS coupled with PEG-20K or dextran T40 at K173C showed alleviated systemic anaphylaxis reaction. Overall, the strategy of site-directed coupling of PEG or dextran is useful for the identification of antigenic determinants, reducing immunogenicity and prolonging plasma half-life of pharmacologically important proteins.

T4

VALPROIC ACID, AN HDAC INHIBITOR, DIFFERENTIALLY INDUCES AUTOPHAGY AND CELL CYCLE ARREST IN HUMAN PROSTATE CANCER CELLS THROUGH DISTINCT EXPRESSION PROFILES OF AUTOPHAGY- AND CELL-RELATED GENES

Xian-Hui He, Dong-Yun Ouyang, Li-Hui Xu Yan-Ting Zhang, Long-Hui Zeng and Shuai Ren
Department of Immunobiology; Jinan University; Guangzhou 510632, China

Valproic acid (VPA), a histone deacetylase inhibitor, has shown promising anti-prostate cancer activities. However, cellular responses including cell proliferation and autophagy to VPA treatment may vary greatly among different prostate cancer cells, which have not been well characterized. In this study, we showed that human prostate cancer LNCaP cells were more sensitive to VPA compared with DU145 and PC-3 cells. Cell cycle inhibitor p21Cip1 was constitutively expressed in LNCaP cells but not in DU145 and PC-3 cells, and was moderately upregulated by VPA in all three cell lines. Some cyclins were differentially depressed by VPA in these cells, resulting in distinct cell cycle arrest. Interestingly, both conversion of LC3-I to LC3-II and formation of LC3 puncta were observed in LNCaP and PC-3 cells but entirely undetectable in DU145 cells, indicating a defect of autophagy in the latter cells. Among several critical autophagy-related proteins, ATG5 and ATG12-ATG5 conjugates, which are essential for autophagy induction, were undetectable in DU145 cells. No canonical transcripts for full length ATG5 but only two alternatively spliced ATG5 transcripts were identified in DU145 cells. These alternative transcripts are lack of one or two exons, leading to premature termination of ATG5 translation. Moreover, transfection of wild-type ATG5 gene into DU145 cells rescued the production of ATG5 and ATG12-ATG5 conjugates, resulting in LC3-II formation. Collectively, these results indicate that VPA-induced autophagy in prostate cancer cells depends on ATG5 and importantly, autophagy pathway is genetically impaired in DU145 cells, suggesting caution in interpreting autophagic responses from this cell line.

T5

PHYSIOLOGIC GENOMICS OR GENOMIC PHYSIOLOGY: THE CHALLENGE AND OPPORTUNITY IN THE GENOMIC ERA.

Nelson Tang
Laboratory for Genetics of Disease Susceptibility, Li Ka Shing Institute of Health Sciences, and KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research of Common Diseases, The Chinese University of Hong Kong.

Forward Genetics and Reverse Genetics are now becoming historical terminologies which essentially describe the relationship between functions / phenotype and genetic variations. In the past decade, starting from the completion of Human Genome Project, there has been a spectacular revolution in technology which allows determination of genotypes in a massive scale. Today, I will present some work in relating Genome to Physiology and hope to convince you that this is a field of good opportunity.

First, I will first illustrate with an example of how Genome-wide association study (GWAS) was used to identify the mechanism of adaptation to survival in high altitudes (This is the work done by Prof. Michael Tam). Then, I will discuss how functional studies could be used to examine the role of genetic polymorphisms in regulation of gene expression or hormone production, using an example of IGF1. Other studies on variation and regulation of transcription in the human population will also be presented.

T6

P2Y RECEPTORS IN HUMAN AIRWAY EPITHELIA: FROM ION TRANSPORT TO AIRWAY INFLAMMATION

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P2Y receptors are expressed in apical and/or basolateral membranes of virtually all polarized epithelia to control the transport of fluid and electrolytes1. In asthmatic inflammation, the bronchial epithelia will be damaged by eosinophil-derived, highly toxic cationic proteins, such as major basic protein (MBP). Therefore, extracellular nucleotides, such as UTP and ATP, would be released into the extracellular space from airway epithelial cells and act in an autocrine or paracrine fashion to regulate ion transport processes and immune functions.

In the human bronchial epithelial cell, 16HBE14o-, the selective P2Y6 receptor agonist, UDP, could stimulate transepithelial Cl- secretion via both Ca2+- and cAMP- dependent pathways2. In an established cell model of asthmatic inflammation3, damage to the airway epithelia by cationic proteins, poly-L-arginine, induced both ATP and UDP/UTP release into the extracellular medium. Moreover, the activation of different P2Y receptor subtypes by specific agonists led to the production of at least two pro-inflammatory cytokines, interleukin (IL)-6 and IL-8. Therefore, the nucleotides that are released during these processes will activate multiple P2Y receptors, which will lead to the further release of inflammatory cytokines. The secretion of cytokines and the formation of such "cytokine networks" play an important role in sustaining the airway inflammatory disease.

The work was supported by a Direct Grant for Research, The Chinese University of Hong Kong (#2041539), and Research Grant Council General Research Fund (#2140595 and #2140730) awarded to W. H. Ko.

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Session II: 15:00-15:50, 16:20-18:00, 14th June

T7

CALCIUM DYSREGULATIONS AND AUTOPHAGY IMPAIRMENT IN FAMILIAL ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a common form of dementia that involves slowly developing and ultimately fatal neurodegeneration. The etiology of AD is unknown, however, the inherited cases of familial AD (FAD) caused by the mutation in presenilins (PS1, PS2) share the same neuropathological hallmarks with the sporadic AD. Their consistent phenotypes suggest they may share the same pathophysiological origins. Thus, insights into the molecular mechanisms and cellular functions of mutant PS associated with FAD are likely to provide clues into the etiology of AD.

Several hypotheses have been proposed to describe the pathogenic mechanisms for FAD. While accumulation of amyloidogenic Ab plaques is a well-defined proximal feature that causes neural toxicity leading to brain pathology, another hypothesis suggests that dysregulation of calcium (Ca^{2+}) homeostasis plays a central role in AD pathogenesis. A considerable body of evidence has demonstrated that FAD-linked PS mutations affect cellular Ca^{2+} homeostasis. We have demonstrated a molecular interaction between inositol trisphosphate receptor ($InsP_3R$) and PS where mutant PS exerted stimulatory effect on $InsP_3R$ to enhance its channel activity in response to $InsP_3$ and resulting in exaggerated Ca^{2+} release. However, how disrupted Ca^{2+} homeostasis impinges on AD pathology is still largely unknown. Recently, we identified a molecular interaction between PS1 and lysosomal two-pore channel (TPC2). FAD-linked PS1 mutation (PS1-M146L) disrupted the lysosomal Ca^{2+} homeostasis and thereby deranged the acidification of lysosome. The resulting increased lysosomal pH could lead to the accumulation of

autophagic vacuoles and may suggest a novel molecular mechanism for autophagic pathology in AD.

T8

THE ROLE OF MIR-196A IN CELL AND TRANSGENIC MOUSE MODELS OF HUNTINGTON'S DISEASE

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Huntington's disease (HD) is a genetic disease caused by an expansion of CAG repeats in the exon 1 of *huntingtin(htt)* gene, leading to neurodegenerative symptoms. To date, there is no cure for HD. Based on previous studies, transcriptional dysregulation plays a critical role in pathogenesis of HD, especially that microRNA(miRNA) may involve in the progression of HD. Here, we demonstrated the potentially therapeutical function of one specific miRNA, mir-196a, on HD via cell and transgenic mouse models. In the *in vitro* results, as mir-196a was overexpressed, reduction of mutant htt was observed in HD models of human embryonic kidney cells and mouse neurobalstoma cells, further decreasing the pathological formation of mutant htt aggregates. In the *in vivo* transgenic mouse model, HD transgenic mice overexpressing mir-196a revealed the suppression of mutant htt expression in brain regions, improving the neuropathological progression, such as decrease of intranuclear and neuropil aggregates at late stage. Furthermore, behavioral motor dysfunction of HD transgenic mice was significantly rescued through overexpressing of mir-196a when these mice were examined using rotard rod, grasping and clasping tests. Advanced mechanism of mir-196 functions on HD was investigated, showing mir-196 alleviated HD symptoms through the alternation of astrocyte and neurotrophic factors expression as well as the functions of ubiquitin-proteasome systems *in vivo*. This study suggests the important role of mir-196a in HD, and provides a new insight of therapeutical strategy for HD.

T9

RADIATION-INDUCED INCREASE IN CELL MIGRATION AND METASTATIC POTENTIAL OF CERVICAL CANCER CELLS OPERATES VIA THE K-Ras PATHWAY

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Radiotherapy is a well established treatment for cervical cancer, the second most common cancer in women worldwide. However, metastasis often circumvents the efficacy of radiotherapy. This study was conducted to elucidate the molecular mechanism of radioresistance-associated metastatic potential of cervical cancer cells. We established three radioresistant cervical cancer cell lines by exposure of cells to a sublethal dose of radiation and screened for lines that exhibited an increased migration phenotype for at least 6 months before undertaking mechanistic studies. Radiation-associated metastatic potential was evaluated using a wound-healing assay, time-lapse recording, and cell locomotion into the lungs of BALB/c nude mice. The radioresistant C33A and CaSki cell lines, but not the radioresistant HeLa cell line, exhibited significantly increased cell migration and wound healing than did wild-type cells. Furthermore, K-Ras played a prometastatic role via the activation of c-Raf/p38, whereas interference of those mediators via either RNA interference-mediated knockdown or the use of chemical inhibitors substantially reversed the radioresistance-associated increase in cell migration. Clinical examination further showed the relative upregulation of the K-Ras/c-Raf/p38 pathway in locally recurring tumors and distant metastases compared with in the primary cervical tumor. These findings demonstrate that a sublethal dose of radiation can enhance the metastatic potential of human cervical cancer cells via K-Ras/c-Raf/p38 signaling, highlighting the potential development of specific inhibitors for reducing metastatic potential during radiotherapy.

T10

UROCORTIN REDUCES CEREBRAL HEMORRAGE INJURY PARTIALLY VIA PI3K/Akt AND GSK-3β SIGNALING PATHWAYS

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Intracerebral hemorrhage (ICH) remains a serious clinical problem lacking effective treatment. ICH frequently causes brain edema, which leads to an expansion of brain volume and worsens ICH outcomes. Increasing evidence suggests that inflammatory cascades are involved in ICH-induced brain edema. Urocortin 1 (UCN), a member of the corticotropin-releasing factor family, protects ischemic cardiomyocytes and dopaminergic neurons. In this study, we examined the therapeutic effect of UCN on ICH-induced neurological deficits and neuroinflammation. ICH was induced in male Sprague-Dawley rats by intrastriatal infusion of bacterial collagenase VII-S or autologous blood. At 1 h after the induction of ICH, UCN either administered intracerebroventricularly (5μg, icv) or intraperitoneally (2.5 or 25 μg/kg) reduced neurological deficits from 1 to 7 days post-ICH. Surprisingly, although a higher dose (25 μg/kg, i.p.) also reduced the functional deficits associated with ICH, it is significantly less effective than the very low dose (2.5 μg/kg, i.p.). Beneficial results with the low dose of UCN also included a reduction in brain edema, BBB disruption, lesion volume, microglial activation and neuronal loss 3 days post-ICH, and suppression of TNF-a, IL-1β, and IL-6 production 1, 3 and 7 days post-ICH. Systemic post-ICH treatment with UCN reduced striatal injury and neurological deficits, likely via suppression of microglial activation and inflammatory cytokine production. We further demonstrated that the fluorescently label-UCN penetrated more prominent into the neurons than microglia, but not astrocytes. Both PI3K/Akt and GSK-3β signaling pathways participate in the UCN-mediated protection against ICH-induced brain injury. Our data suggest that the neuroprotective mechanism of UCN may involve GSK 3β inhibition through phosphorylation on Serine 9 of GSK3β in ICH rats. (*Supported by NSC 99-2320-B-038-006-MY3, Taiwan)*

T11

BURSTING ACTIVITY OF THE CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR REVEALS INTRINSIC GATING SCHEME

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The cystic fibrosis transmembrane conductance regulator (CFTR) is an anion channel defective in the genetic disease cystic fibrosis (CF). Channel openings and closings, termed channel gating of CFTR is characterized by bursts of openings interrupted by brief shuts and separated by long closures between bursts. It is unclear how these short-lived shuts and sustained openings occur during CFTR bursting state and whether CF-associated mutations disrupt their properties. We address these questions by studying single-channel properties of CFTR at pH_i 7.3 and 6.3. Compared to neutral, acidic pH_i altered CFTR gating at bursting state with decreased open time constant t_o and increased fast closed time constant t_{cf}. These alterations were not sensitive to ATP concentrations but nicely simulated by the $C_1 \leftrightarrow O \leftrightarrow C_2$ kinetic scheme. Moreover, studying CFTR variants demonstrates that the acid-sensitive alteration in t_o was abolished by site-directed mutations at two ATP binding sites (site 1 and site 2) in CFTR. In contrast, t_{cf} regulation by acidic pH_i was altered by mutants at site 1 but not site 2. CF mutant G1349D-CFTR at site 1 markedly reduced the value of t_{cf} and t_o, whereas CF mutants G551D-CFTR at site 2 and DF508-CFTR showed small or no effects on them. Our data suggest that site 1 predominantly controls gating events during bursting state. This study provides a fundamental basis of CFTR gating mechanism suggesting how CF-associated mutations affect CFTR gating.

T12

DEFUNCT BAROREFLEX RESPONSES UNDERLIE CARDIOVASCULAR COLLAPSE ASSOCIATED WITH METHAMPHETAMINE INTOXICATION

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The psychostimulant methamphetamine (MTEH) possesses high potential for abuse and addiction because it heightens alertness and energy, induces euphoria, enhances self-respect and increases sexual pleasure. At issue is that METH intoxication is a common cause of death within the abuse population, although the underlying mechanism is still unsettled. This presentation summarizes our recent work on the cellular mechanisms that underpin cardiovascular collapse associated with METH intoxication, using adult, male Sprague-Dawley rats.

Intravenous administration of METH (12 or 24 mg/kg) induces a time-dependent and dose-dependent distribution of the psychostimulant in brain and heart. Of note is that the distribution of METH to neural substrates associated with the baroreflex loop is significantly larger than brain targets for its neurological and psychological effects; the concentration of METH in cardiac tissues is the lowest. High doses of METH induce significant mortality within 20 min that parallel concomitant collapse of arterial pressure or heart rate, with defunct baroreflex-mediated sympathetic vasomotor tone and cardiac response. These events are accompanied by concurrent increases in the concentration of METH in serum and the rostral ventrolateral medulla (RVLM), along with tissue anoxia, cessation of microvascular perfusion and necrotic cell death in this key component of the baroreflex loop. Furthermore, mitochondrial respiratory chain enzyme activity or electron transport capacity and ATP production in RVLM are reduced, and mitochondria-derived superoxide anion level is augmented. All those detrimental physiological and biochemical events are reversed on microinjection into RVLM of a mobile electron carrier in the mitochondrial respiratory chain, coenzyme Q10; a mitochondria-targeted antioxidant and superoxide anion scavenger, Mito-TEMPO; or an oxidative stress-induced necrotic cell death inhibitor, IM-54.

We conclude that on intravenous administration, METH exhibits a preferential distribution to key brain stem nuclei associated with the baroreflex circuit. Furthermore, sustained anoxia and cessation of local blood flow that leads to bioenergetics failure and oxidative stress because of mitochondrial dysfunction, leading to acute necrotic cell death in RVLM underpins the cardiovascular collapse because of defunct baroreflex responses elicited by lethal doses of METH.

Session III (Hong Kong-Guandong Biophysics Symposium): 11:00-12:45, 15th June

T13

AUTONOMOUS FORMATION OF SEQUENTIAL PERIODIC STRIPES FROM DENSITY-DEPENDENT MOTILITY

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Sequential and periodic patterns are recurring anatomical features in living organisms. Their rhythmic dynamics and intriguing beauty have fascinated generations of scientists. However, the understanding of the underlying mechanisms is hindered by the overwhelming molecular complexities in most cases. Engineered synthetic systems can simplify the complexities and refine the theoretical assumptions, thereby providing insights into the principles of naturally occurring phenomena. Here we described a synthetic pattern formation system by simply coupling cell density and motility, which enabled the programmed cells to form crisp, periodic stripes of high- and low- densities in a sequential and autonomous manner. Theoretical and experimental analyses revealed that the periodic structure arises from a recurrent aggregation process generated during the continuous expansion of the cell population. In accordance with our model prediction, patterns with different numbers of stripes were generated by tuning the activity of a single promoter. The results establish motility control as a simple, potent route for generating regular spatial structures without the need of a pacemaker, and illustrate the utility of synthetic genetic systems in studying pattern formation in spatially extended systems.

T14

CHOLECYSTOKININ: THE MEMORY-WRITING CHEMICAL IN THE BRAIN

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Patients with damage to the hippocampal system have great difficulty forming new long-term declarative memories, but still recall their remote memories, while neocortical damage impairs remote memories. This paper presents direct evidence of the establishment of artificial visuoauditory memory traces in the rat auditory cortex and the participation of the entorhinal cortex in the establishment and retrieval of these memory traces. We produced an association between an artificial cortical activation and a visual stimulus with classical conditioning. The memory traces were physiologically visualized from auditory neuronal responses to the visual stimulus after conditioning and behaviorally confirmed with a memory recall experiment. Formation of a new artificial visuoauditory memory in the auditory cortex with classical conditioning was bilaterally blocked when the entorhinal cortex was unilaterally temporarily inactivated, but easily achieved if the entorhinal cortex was not inactivated. We found that cortical projection neurons in the perirhinal and entorhinal cortices were cholecystokinin (CCK)-immunopositive. CCK application in the auditory cortex of anesthetized rats enabled the cortical neurons to respond to a previously ineffective tone stimulus, after the tone had been paired with the best-frequency tone stimulus. It also enabled the cortical neurons to respond to a light stimulus after the stimulus had been paired with a strong noise-burst stimulus. Further, in-vivo intracellular recordings in the auditory cortex showed that synaptic strength was potentiated after 2-trial pairing of presynaptic and postsynaptic coactivities in the presence of CCK. An application of a CCK receptor antagonist in the auditory cortex prevented the formation of the visuoauditory associative memory by classical conditioning in behaving rats, similarly to the entorhinal cortex inactivation experiment. We conclude that the hippocampal system exerts its influence on cortical neuroplasticity through the action of CCK. Similar to the WRITE-ENABLED switch for memory writing in a computer, CCK switches the memory writing in the neocortex.
Supported by HK RGC (5610/09M and PolyU9/CRF09)

T15

STRUCTURE-FUNCTIONAL STUDY OF PROTEINS THAT REGULATE CELL PROLIFERATION AND DIFFERENTIATION

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Proper organ development requires the precise regulation of both the total number of cells (cell proliferation) and the types of cells (cell differentiation). During cell proliferation, Cdt1 mediated loading of DNA helicase (Mcm2-7) to replication origins is required for DNA replication. And Hox gene activation is necessary for embryonic cell differentiation. It has been shown that these two processes are linked through the cell cycle-regulator Geminin and the homeodomain-containing transcription factors Hox. To understand the molecular mechanism involved, we determined the solution structures of Geminin-Hox and Cdt1-Mcm6 complexes by nuclear magnetic resonance (NMR) spectroscopy and conducted biochemical study to delineate the structural basis of this mutual regulation. In addition, we found that histone H4-K20 methyltransferase SET8 is a new cell-cycle regulator and plays an important role in the developmental program of metazoans. (*These works are supported by RGC GRF grants 663911 and 664109, and by TUYF.)*

T16

BIOPHYSICAL PROPERTY OF HUMAN RED BLOOD CELL AS A FUNCTION OF CELL AGING AND PATHOLOGICAL CONDITIONS

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The biophysical property of human red blood cell (RBC) was investigated as a function of cell aging and

some pathological conditions. The studied sample cells include: (1)Normal human RBCs with different cell ages (young RBC, senescent RBC or old RBC and two middle age RBCs fractionated by Percoll-centrifugation) ; (2) Normal human RBCs stored in blood bank for different periods (one day, four days, one week, two weeks, three weeks and four weeks); (3) Human RBCs in abnormal physiological conditions (abnormal temperature, pH and sickness such as anemia). (4) Human RBCs under the exposures of non-ironization radiation. The biophysical properties determined in the research including the morphological parameters, the bending modulus and shearing modula, the deformability, the surface sialic acid and charge density, the ATP and DPG contents of the cells. It was found that both the membrane moduli increased as cell aging and storage time, whereas the deformability of the cells decreased. In the variation of the biophysical properties with temperature, both the bending modulus and shearing modulus of the cells have minimum values at 37 °C, but increased as the temperature was away from the normal value. Similar situation was found in the variation of the biophysiical properties with pH condition, both the bending modulus and shearing modulus of the cells have minimum values at pH 7.4 , but increased as the environmental pH was away from the normal one. The anemia RBC and the RBC under radiation exposures were found to be of greater bending and shearing moduli than normal cell and more difficult to get through capillary. The variation of the properties of these huamn RBCs was found accompanied with decreases in cell surface sialic-acid *N*-acetylneuraminicacid[NANA] (surface charge), ATP and DPG contents, and beleived to be a result of lossing these biological molecules and materials from the cells.

Session IV: 16:15-18:00, 15th June

T17

MODELING FM RESPONSES OF MIDBRAIN AUDITORY NEURONS WITH COMPLEX RECEPTIVE FIELDS

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Frequency modulation (FM) is an important building block of communication signals for mammals including human. Attempts to model the response of central neurons to FM sounds have been in general unsatisfactory, despite the possible insights of modeling brought on the underlying neural mechanisms. Here we extended a previous approach on modeling FM neurons with simple receptive field to those neurons with complex receptive field. We started with single unit data in the anesthetized rats obtained in response to a random FM tone. The spectro-temporal receptive fields (STRF) and peri-stimulus time histogram (PSTH) were then generated. For each neuron, we grouped peaks in the PSTH based on the similarity in trigger feature (modulation speed and spectral extent). For each group, the temporal window and its delay time to spike occurrence were optimized in modeling the PSTH using a finite impulse response neural network (FIRNN). Finally we tested the performance of the composite model (mixture of experts) in training and predicting the responses. We found performance level markedly exceeding those based on simple features. Results were taken to support the importance of trigger features, regardless of their complexity, in predicting FM responses in the auditory midbrain. (Supported by National Science Council, Taiwan.

T18

MOLECULAR MECHANISM OF CIRCUIT FORMATION IN THE RODENT SOMATOSENSORY BARREL CORTEX

Tomomi Shimogori

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Dynamic morphological changes of dendrites and synapse formation are one of the most remarkable features of the developing brain, and make it possible for neurons to change their connectivity depending on stimuli from the outside world and to adapt behavior. To elucidate the molecular mechanism of activity dependent circuit formation, we used rodent somatosensory barrel cortex, which development

occurs in their first postnatal week. In the barrel cortex, thalamocortical axons (TCAs) from individual thalamic barrelloids are almost entirely confined to single barrel clusters, followed by arrangement of cortical layer IV neurons into barrel hollows and septa. Addition to this, unidirectional dendrite formation of barrel neurons toward barrel hollows occurs for efficient synapse formation with TCAs. Using Allen's brain atlas and our own microarray study, we isolated candidate molecules, which mediate activity dependent barrel formation. Moreover, gain and loss of function demonstrated that when and how these molecules are involved in barrel development. Our results provide the molecular framework of barrel cortex development, which is controlled by neuronal activity via thalamocortical axon.

T19

HELPING ISOLATED NEURONS MAKE APPROPRIATE CONNECTIONS

G. W. Arbuthnott, W. A. Staines, C. Vickers, F. E. Randall, S. C. Schock, M. Garcia-Munoz
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The corticostriatal system has been suggested to be the seat of motor learning and habit formation. We hoped to be able to study the properties of this simple two-neuron circuit in culture. Because the characteristic neuronal morphology is not well developed in culture we used different mouse lines. One of the groups of neurons (usually cortex) came from a mouse genetically modified to express GFP in all cells and the other from a normal mouse line.

It was relatively easy to find pairs of neurons and to study the properties of the synaptic connections. Of the pairs recorded 45% were not connected. In total 32 pairs were connected by the expected excitatory synaptic connections from cortex to striatal neurons. However, 53 pairs were connected in the striatal to cortical direction. This connection never forms in brains.

We have developed a better preparation and have been able to show that this time the connections are more appropriate. Cortical and striatal neurons are plated in two compartments separated by a 500μm barrier that is removed after the neurons have attached to the substrate. Cortical neurons grow connections across the gap to reach striatal neurons. The striatal cell activity recorded on multielectrode arrays is driven by cortical activity and the striatal activity is silenced by cutting the connections between the groups of neurons. If we infect the cortical cells with Channel Rhodopsin2 then the striatal cells are driven by the light induced activity in the cortical neurons. When the striatal cells are similarly infected, we have not seen any response in cortical neurons.

This method may also be useful in other situations where the study of individual pairs of connected neurones is difficult, although exactly how similar such simplified systems are to those in brain needs careful study.

T20

HIPPOCAMPAL NEUROGENESIS FOLLOWING TRAUMATIC BRAIN INJUTY

Yi-Ling Yang
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Adult neurogenesis occurs in the subgranular zone of the hippocampal dentate gyrus, and can be modulated by physiological and pathological events. We examined the effect of vascular endothelial growth factor (VEGF), and the correlation between VEGF and the Raf/MEK/ERK cascade in neurogenesis after traumatic brain injury (TBI). The expression of VEGF and the phosphorylation level of Raf/MEK/ERK were analyzed by Western blot, and TBI-induced neurogenesis was determined by immunofluorescence labeling and confocal microscopic detection. Hippocampal VEGF began to increase after 12 h, and reached a peak at day 7. Along with the upregulation of VEGF, neurogenesis in the hippocampus also increased. Administration of the VEGF antisense oligodeoxynucleotide, or the VEGF receptor-2 antagonist SU1498 (10 mg, ICV), attenuated the phosphorylation of the MAPK cascade proteins and caused a decrease in neurogenesis in the hippocampus. Similarly, administration of the ERK inhibitor PD98059 (500 ng, ICV) also exhibited a suppressive effect on neurogenesis. Our results indicate that VEGF plays an important role in neurogenesis after TBI, and that the process involves VEGF receptor-2 and the Raf/MEK/ERK cascade.

Young Investigators Symposium

Session I: 9:30- 10:30, 15th June

Y1

REGULATION OF STABLE NERVE-MUSCLE INTERACTION AND SYNAPTOGENSIS AT THE NEUROMUSCULAR JUNCTION BY PTEN SIGNALING.

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During the development of the vertebrate neuromuscular junction (NMJ), growing motor axons come into contact with their muscle targets and differentiate into presynaptic nerve terminals specialized in the release of neurotransmitter acetylcholine (ACh). In cultured Xenopus nerve-muscle cocultures, the presynaptic development is preceded by the cessation of axonal growth upon muscle contact. In this study, we examined the function of presynaptic PTEN (phosphatase and tensin homolog) in mediating this growth-to-synaptogenesis transition of spinal neurons. Application of bpV (potassium bisperoxo [1,10-phenanthroline] oxovanadate; phen), a potent PTEN inhibitor, rendered spinal neurons to "overlook" their muscle target, disrupted stable nerve-muscle interaction and inhibited NMJ assembly. Consistently, similar results were obtained by knocking down PTEN through introducing into spinal neurons antisense PTEN Morpholino oligonucleotides or overexpression of C124S-PTEN (a catalytically dead form of PTEN). Taken together, these results suggest a novel function of PTEN in regulating stable contacts between synaptic partners and NMJ assembly. (Supported by Hong Kong RGC GRF grants 662311 and 662108)

Y2

EXPRESSION OF TOLL-LIKE RECEPTOR 4 SIGNALLING COMPONENTS IN ADULT RAT DORSAL ROOT GANGLION CELLS

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Aim: To investigate the expression of toll-like receptor 4 (TLR4) in dorsal root ganglion (DRG) cells. Methods: DRG cells were isolated from adult SD rats, and neuron-enriched (N-cell) and glial cell (G-cell) cultures were analysed using a RT2 ProfilerTM PCR Array for rat toll-like receptor signalling pathways. CD14, MD-1, MD-2, RP105 and TLR4 mRNA was extracted using Trizol and analysed by RT-PCR and/or qPCR using TaqMan probes. CD14, MD-1, MD-2, RP105 and TLR4-immunoreactivity in DRG cell cultures were visualized by confocal microscopy. Results: TLR4, TLR5 and TLR9 were predominantly expressed in neuron-containing cell cultures, with TLR4 showing the highest expression relative to β-actin mRNA. TLR4 and its co-receptors (CD14, MD-1, MD-2, RP105) and adaptors (MyD88, TRIF) were expressed in both N-cells and G-cells. qPCR confirmed that TLR4 mRNA expression was higher in neuron-containing cultures than pure glial cell cultures. CD14, MD-1, MD-2, RP105 and TLR4-ir were only localized on DRG neurons but not glia. Quantification showed the percentage of TLR4+/CD14+ or TLR4+/MD-2+ neurons were higher than those of TLR4+/RP105+ or TLR4+/MD-1+ neurons. Conclusions: Messenger RNA of TLR4 and its signalling components was detected in both DRG neuron-enriched cultures and in DRG glial cell cultures, but protein expression was only evident on DRG neurons. Further investigations are ongoing to determine the functional role of neuronal TLR4 in sensory ganglia. Acknowledgements/disclosures: This work was supported by a grant from the Research Grants Council of the Hong Kong SAR (GRF476710).

Y3

PROTECTIVE EFFECTS OF LYCIUM BARBARUM POLYSACCHARIDES ON CEREBRAL EDEMA AND BLOOD-BRAIN BARRIER DISRUPTION AFTER ISCHEMIC STROKE

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Background: Ischemic stroke is a destructive cerebrovascular disease and one of the leading causes of death worldwide. The long term disability after stroke induces heavy burden both to the patients and the society. Yet, no effective neuroprotective agents are available. The polysaccharides extracted from the fruits of wolfberry, Lycium barbarum (LBP), showed neuroprotective and immune-modulative functions. We aim to evaluate the protective effects of LBP in experimental stroke using a focal cerebral ischemia/reperfusion (I/R) model. Methods: C57BL/6N mice were subjected to 2 h of middle cerebral artery occlusion (MCAO) followed by 22 h of reperfusion. Prior to ischemia induction, animals were treated with either vehicle (PBS) or LBP daily for 7 days. Mice were evaluated for neurological deficits just before sacrifice. Brains were harvested for infarct size estimation, water content measurement and immunohistochemical analysis as well as Western blot experiments. Evans blue (EB) extravasation experiment was performed to determine blood-brain barrier (BBB) disruption after MCAO. Results: LBP treatment significantly improved neurological scores and decreased infarct size, hemispheric swelling and water content as well as reduced EB extravasation. In addition, fewer apoptotic cells were identified in the LBP-treated brains by TUNEL assay. Immunoreactivity for aquaporin-4 and glial fibrillary acidic protein were also significantly decreased in LBP-treated brains. We further observed a reduction of nuclear factor-κB translocation and IκB expression after LBP treatment. Conclusion: Seven-day LBP pre-treatment effectively improved neurological deficits, decreased infarct size and cerebral edema as well as protected the brain from BBB disruption, aquaporin water channel up-regulation and glial activation. The protective effects of LBP might partially act through its anti-inflammatory effects. The present study suggests that LBP may be used as a preventive neuroprotectant for ischemic stroke.

Y4

GENOME-WIDE EXPRESSION ANALYSIS OF MIGRATORY SACRAL NEURAL CREST CELLS FROM THE DOMINANT MEGACOLON MOUSE MUTANT

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Hirschsprung's disease (HSCR) is a congenital disease which is found in approximately 1 in every 5000 live births. It is characterized by the reduction or absence of neurons and glial cells of the enteric nervous system in the gut. Failure of neural crest cells (NCCs) to colonize the gut during the embryonic development has been considered as one of the possible causes of the disease. In this study, we analyzed the gene expression profile of migrating sacral NCCs of the spontaneous mouse mutant *Dominant megacolon (Dom)* which is a HSCR animal model expressing a mutated transcription factor Sox10. Delayed migration and aggregation of sacral NCCs from the explanted neural tube of homozygous embryos were found *in vitro*. Gene expression profiling of these migrating sacral NCCs with microarray analysis demonstrated changes of expression of a number of genes including those involved in myelination, melanogenesis and adhesion. Three of the adhesion molecules, which have been proposed to play important roles in initiation and cessation of NCC migration, were found to be significantly down-regulated. One of them was further characterized using luciferase reporter assays and chromatin immunoprecipitation. It was found that Sox10 activated the adhesion molecule *in vitro* through a specific binding site on the adhesion molecule. Hence our results implicate that the mutation of Sox10 in the *Dom* mutant down-regulates the expression of the adhesion molecule leading to the abnormal migration of sacral neural crest cells.

Acknowledgements: This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project no. CUHK461808).

Y5

A NOVEL MECHANISM OF CONTROL OF NFκB ACTIVATION AND INFLAMMATION INVOLVING A_{2B} ADENOSINE RECEPTORS

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The NFκB pathway controls a variety of process, including inflammation, and thus, the regulation of NFκB has been a continued focus of study. Here, we report a newly identified regulation of this pathway, involving direct binding of the transcription factor NFκB1/p105 to the C-terminus of the A_{2B} adenosine receptor (A_{2B}AR), independent of ligand activation. Intriguingly, binding of A_{2B}AR to specific sites on p105 prevents polyubiquitination and degradation of p105 protein. Ectopic expression of the A_{2B}AR increases p105 levels and inhibits NFκB activation, while p105 protein levels are reduced in cells from A_{2B}AR knockout mice. In accordance with the known regulation of expression of anti- and pro-inflammatory cytokines by p105, A_{2B}AR null mice generate less IL-10, and more IL-12 and TNF-α. Taken together, our results show that the A_{2B}AR inhibits NFκB activation by physically interacting with p105, thereby blocking its polyubiquitination and degradation. Our findings unveil a surprising function for the A_{2B}AR, and provide a novel mechanistic insight into the control of the NFκB pathway and inflammation.

Y6

EFFECT OF A MUSCARINIC RECEPTOR AGONIST AND A NITRIC OXIDE SYNTHASE INHIBITOR ON THE REGULATION OF GASTRIC MYOELECTRICAL ACTIVITY IN ICR MICE

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Background: Gastric myoelectrical activity (GMA) is composed of slow waves and spike activity. Slow waves originate from interstitial cells of Cajal located within enteric nervous system and play an important role in controlling the frequency and direction of gastrointestinal contractions. GMA can be detected by electrogastrography. The aim of the present study is to investigate the effects of muscarinic receptor agonist, bethanechol, and the nitric oxide synthase (NOS) inhibitor, L-NAME, on gastric slow wave activity in mice.

Methods: Male ICR mice (3 month old, 30-40 g) were anaesthetised and surgically implanted with telemetry devices (PhysioTel® ETA-F20, DSI, U.S.A.) with recording wires sutured into the serosal side of the stomach. After 7 days recovery, animals were randomised to receive vehicle (saline 2 ml/kg, i.p.), bethanechol (6 mg/kg, i.p.), or L-NAME (10 mg/kg, i.p.). Two hours of baseline GMA was recorded prior to drug/vehicle administration; recordings then continued for a further 6 h. Raw data were processed using Spike2 (Cambridge Electronic Design, U.K.) and analysed using a repeated measures 2-way ANOVA followed by Bonferroni t-tests.

Results: In the bethanechol session, the baseline dominant frequency (DF) of the vehicle (n=8) and treatment (n=7) groups was 6.9 ± 0.7 counts per minute (cpm) and 6.6 ± 1.0 cpm, respectively. In the L-NAME session, the baseline DF of the vehicle (n=7) and treatment (n=7) groups was 6.7 ± 0.7 cpm and 7.1 ± 0.7 cpm, respectively. Saline had no effect on any of the parameters of the slow waves ($P>0.05$). Similarly, the DF did not alter after treatment with bethanechol or L-NAME ($P>0.05$). No significant changes were observed in the % power of bradygastria, normogastria, or tachygastria before and after the drug administration ($P>0.05$).

Conclusion: Muscarinic receptor stimulation and a potential reduced level of NO had no effect on the

DF or the % power distribution of gastric slow wave activity.

Y7

BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) REGULATES THE ACQUISITION OF VESTIBULAR-MEDIATED SPATIAL ORIENTATION IN RATS

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Critical period is a postnatal time window during which an organism displays a heightened sensitivity to certain environmental stimuli. One factor identified to account for the decline in plasticity after critical period is the maturation of inhibitory circuitry. Brain-derived neurotrophic factor (BDNF) has been widely implicated in GABAergic maturation. We have previously demonstrated that neonatal blockade of GABAA receptor in the rat vestibular nuclei delayed the expression of negative geotaxis, a gravity detection behaviour. To attest the effects of BDNF in the acquisition of vestibular reflexes, neonatal rats implanted with BDNF-loaded Elvax slices over the brainstem vestibular nuclei were tested on negative geotaxis. In the control group, young rats were unable to re-orient from an ear-down (transverse orientation) and a nose-down (antero-posterior orientation) to a nose-up position until P7 and P9, respectively. However, chronic exposure of BDNF accelerated the expression of negative geotaxis. Data from whole-cell patch-clamp experiments in central vestibular neurons of neonatal rats also indicated that BDNF is associated with long-term changes in $GABA_A$ receptor-mediated currents. Taken together, our data suggest that developmental acquisition of spatial orientation can be modified by BDNF-mediated refinement of neural networks in the vestibular nucleus.

Y8

NANO-STRUCTURED PLATFORM FOR HIGHLY SELECTIVE, SENSITIVE AND QUANTITATIVE ELECTROCHEMICAL DETECTION OF HYDROGEN PEROXIDE (H_2O_2) AND CLUCOSE IN BIO-MIMETIC MEDIA

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Hydrogen Peroxide (H_2O_2) is one of the endogenously generated reactive-oxygen-species (ROS). It can act as either a cytotoxin or a cellular messenger, depending on the concentration and location of its endogenous generation. Increasing evidence shows that the concentration of H_2O_2 is related to both cell growth and cell apoptosis. In order to study its functional mechanisms and regulation pathways, it is desirable to have methodologies and/or techniques for its detection in a selective, sensitive and quantitative manner and in the presence of many interfering chemical species.

We report here our effort in the development of a nano-structured electrochemical platform and the usage of it in the detection of H_2O_2 and glucose in a bio-mimetic media. This platform contains three functional layers: an analyte- dependent catalyst, a nano-structured filter and a substrate surface for immobilization of biomolecules or other desirable macromolecules. By using nano-structured platinum (Pt) as the catalyst, we were able to measure H_2O_2 by electrochemical oxidation in a broad concentration range, and in a sensitive and quantitative, and selective manner. As a natural extension to the detection of H_2O_2, we developed a glucose sensor by combining the H_2O_2-sensing platform with the immobilization of glucose oxidase (GOD) on the nano-structured surface spatially separated from that of the H_2O_2-catalysts surface. The selective detection of H_2O_2 and glucose in the presence of common interfering chemical species was also studied.

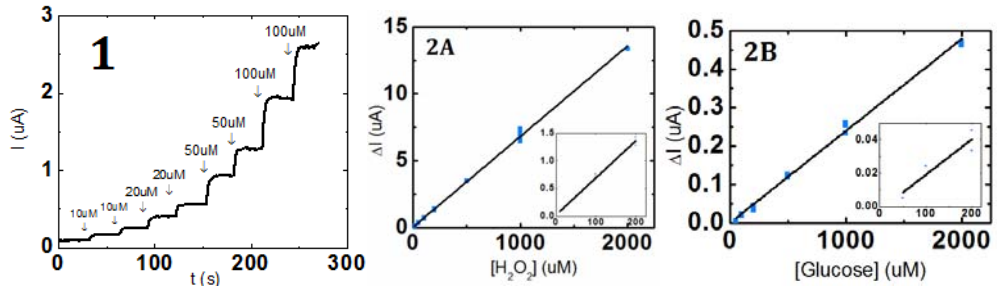

Figure 1: The amperometric i-t curve of addition of different [H_2O_2].

Figure 2: The calibration curve of (A) H_2O_2 and (B) glucose.

Acknowledgement: We acknowledge the support of this project by RGC-HK, WMINST-HKUST, RPC-HKUST, and BIEN PG Program-HKUST.

Poster Presentation

P1- P26 session: 10:20- 11:00, 14th June; P27- P52 session: 15:50- 16:20, 14th June
P53- P78 session: 10:30- 11:00, 15th June; P79- P104 session: 15:45 – 16:15, 15th June

P1

RHO A SIGNALING CONTRIBUTES TO STATIN-INDUCED OSTEOGENESIS IN BONE MARROW MESENCHYMAL STEM CELLS

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Statins, 3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors, reduce cholesterol synthesis and prevent cardiovascular disease. Previous in vitro and in vivo studies showed that statin stimulated bone formation. They also have been found to inhibit prenylation of Rho proteins in recent decade. Previous reports showed that statins inhibited protein prenylation and decreased the active form of Rho A in osteoclasts. Others reports indicated that statins inhibited protein prenylation but increased the active form of Rho A in human erythroleukemia cells. Therefore, the role of statins regulate Rho A activity remains unclear. Rho GTPases act as molecular switches to regulate mesenchymal stem cell differentiation. Previous study showed that transfected constitutively active-form of RhoA into human mensenchymal stem cells (hMSCs) which leaded differentiation of hMSCs into osteoblasts. On the other hand, dominant negative RhoA leaded differentiation of hMSCs into adipocytes. According to the description above, we want to investigate whether Rho A signaling contributes to statin-induced osteogenesis in BMSCs. Pluripotent mesenchymal cells, D1, which were cloned from Balb/c mouse bone marrow cells and purchased from ATCC. For all experiments, cells were treated with or without simvastatin. The mRNA expression of Rho A was detected and quantified by real time PCR. And the mineralization effect on mouse bone mesenchymal stem cells (mBMSCs) was tested by Alizarin Red S Staining. The protein level of active form Rho A was detected by pull down assay. The mineralization showed that the simvastatin were potentially enhanced the cell mineralization on BMSCs. The mRNA expression of Rho A showed that simvastatin 1uM significantly increased Rho A gene expression on first day. However, there were no significantly different between control and treatment group on third and fifth days. The protein level of active form Rho A increased with 1uM simvastatin treatment on the second day. From these results, we suggest Rho A signaling may contribute to simvastatin-enhanced osteogenesis in mBMSCs.

P2

THE REGENERATION OF FULL-THICKNESS ARTICULAR CARTILAGE DEFECT WITH HADSCS LOADED HA/FIBRIN GEL IN A PORCINE MODEL

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Introduction: The self-repaired capability of articular cartilage is limited; moreover, cartilage injuries may result in osteoarthritis. Nowadays, tissue engineering is an alternative way to restore cartilage defects. Our previous in vitro study indicates that hyaluronic acid (HA)/fibrin gel increased chondrogenic gene expressions and sulfated glycosaminoglycans (sGAG) formation in human adipose-derived stem cells (hADSCs), indicating HA microenvironment is critical for chondrogenic differentiation of ADSCs. In this study, we hypothesize that HA-enriched fibrin gel may induce hADSCs chondrogenesis and thus enhance the repair of porcine full-thickness chondral defects.

Materials and Methods: In the ex vivo study, we created a chondral defect (diameter: 2mm) at the

center of isolated osteochondral disc from porcine femoral condyle. The HA/fibrin/hADSCs, fibrin/hADSCs, HA/fibrin or fibrin gel was implanted in the defects and then cultured for 4 weeks. To evaluate the effect of HA/fibrin/hADSCs in the repair porcine chondral defect using histological analysis. In the in vivo study, a full-thickness chondral defect without penetrating subcondral bone (diameter: 6 mm) was created in the medial condyle of a minipig. HA/fibrin/hADSCs or fibrin/hADSCs was fixed in the defects, and empty group is the control. The minipigs were sacrificed at 12 weeks. To evaluate the effect of HA/fibrin/hADSCs in the repair porcine chondral defect by using histological analysis.

Results: In the ex vivo study, we found that HA/fibrin/hADSCs group showed more GAG formation and have better integration with surrounding tissue than other groups. Moreover, in the in vivo study, our preliminary data showed that defect treated with HA/fibrin/hADSCs have more GAG accumulation compared to empty group.

Concludion: The results demonstrated that HA/fibrin may help the regeneration of articular cartilage defect in the ADSCs-based tissue engineering ex vivo, but we should confirm the results by porcine animal study in the future.

P3

SEVERE EXERCISE AND EXERCISE TRAINING EXERT OPPOSITE EFFECTS ON HUMAN NEUTROPHIL APOPTOSIS VIA ALTERING THE REDOX STATUS

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Neutrophil spontaneous apoptosis, a process crucial for immune regulation, is mainly controlled by alterations in reactive oxygen species (ROS) and mitochondria integrity. Exercise has been proposed to be a physiological way to modulate immunity; while acute severe exercise (ASE) usually impedes immunity, chronic moderate exercise (CME) improves it. This study aimed to investigate whether and how ASE and CME oppositely regulate human neutrophil apoptosis. Thirteen sedentary young males underwent an initial ASE and were subsequently divided into exercise and control groups. The exercise group (n = 8) underwent 2 months of CME followed by 2 months of detraining. Additional ASE paradigms were performed at the end of each month. Neutrophils were isolated from blood specimens drawn at rest and immediately after each ASE for assaying neutrophil spontaneous apoptosis (annexin-V binding on the outer surface) along with redox-related parameters and mitochondria-related parameters. Our results showed that i) the initial ASE immediately increased the oxidative stress (cytosolic ROS and glutathione oxidation), and sequentially accelerated the reduction of mitochondrial membrane potential, the surface binding of annexin-V, and the generation of mitochondrial ROS; ii) CME upregulated glutathione level, retarded spontaneous apoptosis and delayed mitochondria deterioration; iii) most effects of CME were unchanged after detraining; and iv) CME blocked ASE effects and this capability remained intact even after detraining.

P4

ESTROGEN INHIBITS H9C2 CARDIOMYOBLASTS FROM FURTHER DIFFERENTIATION

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Estrogen (E2) prevents cardiomyocytes from apoptosis by decreasing the production of reactive oxygen species and improves ventricular functions of infarcted hearts by increasing cell proliferation of c-kit-positive cells in cardiac tissues. Because retinoic acid (RA) accelerates differentiation of embryonic stem cells by enhancing mRNA expression of MLC-2v (myosin light chain-2v is a cardiomyocytes differentiation marker), the purpose of our study was to investigate whether estrogen enhanced differentiation of H9c2 cardiac myoblasts. In our study, H9c2 myoblast was used as our experimental model. DMSO at 10 nM was a negative control. RA at 10 nM was a positive control. A 24-hr treatment with DMSO did not effect on MTT production, a 48-hr treatment increased cell number, and a 72-hr treatment changed the morphology from flat to bipolar shape in cultured H9c2 myoblasts also shift cell

from S to G1 phase. Compared with DMSO at the same incubation period, RA decreased MTT production, further increased cell number, those shift into S phase, then changed the morphology from flat to bipolar shape, and caused cell fusion together in cultured H9c2 myoblasts, E2 decreased MTT production, further increased cell number, those shift into S phase, but reduced the number of cells with bipolar shape. Our data suggest that E2 at 10 nM may inhibit cardiac myocyte differentiation.

P5

PROTECTIVE EFFECT OF ACTIVATED ESTROGEN RECEPTOR BETA ON PALMITATIC ACID-INDUCED APOPTOSIS IN H9C2 CELLS

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Myocardial infarction causes cardiomyoctye apoptosis and metabolic disturbance with the progression of heart failure. Since lipid accumulation induces lipotoxicity in pancreas b cell and hepatocytes and estrogen exerts cardioprotective roles through activating estrogen receptors (ERs), the purpose of this study is to investigate in H9c2 cell line whether palmitatic acid (PA) treatment induces apoptosis and activation of estrogen receptor (ERb) through DPN-mediated suppression of PA-induced lipotoxicity. Quantitative proteomic analysis showed the decreased amounts of lipolytic enzymes and the increased amounts of glycolytic enzymes after coronary occlusion. However, DPN, an estrogen receptor beta agonist, did not reverse the change in the amounts metabolic enzymes. Interestingly, DPN treatment decreased the amount of apoptosis-induced factor (AIF). Pretreatment with DPN prevented the PA-deceased cell viability. ERb antagonist treatment confirms that the protective effect of DPN on cell viability is ERb-dependent. PA treatment increased the expression of cell cycle and apoptosis regulatory protein-1 (CARP1) and AIF, which are also involved in caspase-independent apoptosis. Pre-treatment with DPN did not affect PA-increased AIF expression but prevented the increase of CARP1 expression. In conclusion, PA treatment mimic lipid accumulation-induced apoptosis in cardiomyocyte and the action of PA-induced lipotoxicity is through CARP1 and AIF. Activation of ERβ prevents PA-induced apoptosis through suppressing CARP1 expression.

P6

THE INFLUENCE OF GENDER DIFFERENCES ON ESTROGEN-INDUCED TUMOR DEVELOPMENT IN 4T1-IMPLANTED MICE

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Breast cancer occurs in both female and male, but it is mainly diagnosed in postmenopause female. The difference in the incidence may be due to the hormone environment. Up to date, both estrogen receptor-α (ERα) and -β (ERβ) are identified in breast tumor tissues of both males and females. Because the induction of ERα (+) tumor growth by estrogen is gender-independent [2], the purpose of this study was to investigate whether estrogen-induced tumor growth of ERβ-containing breast tumor cells is gender-dependent. 4T1 breast cancer cells were implanted into BALB/c mice at the age of 5-week old. After implantation, males, females, and ovariectomized females were treated with estrogen for 2 weeks. In the vehicle controls, tumor densities of male mice were significantly higher than intact female mice. And also the density tumor densities of ovariectomic female mice were higher than intact female mice. With treatment of estrogen, only male mice had significantly larger tumor than vehicle control in 1-week while ovariectomic mice were significantly higher than vehicle controls in 2-week. After 2-week treatment with estrogen, the increases of total density and areas in the male group were counteracted by the co-treatment of PHTTP. The data showed that in male mice, estrogen induced tumor growth in 4T1-implanted mice while PHTTP inhibited the growth. In ovariectomic female mice, estrogen induced tumor growth in 4T1-planted mice, PHTTP didn't inhibit the growth while intact mice had no significant in all groups. The tumor growth of intact female mice was not affected by injected estrogen as compared with ovariectomic mice. Since our preliminary data show that estrogen didn't promote 4T1 growth in cell lines, but induced cell migration, the results suggested that estrogen induces estrogen receptor beta-tumor growth in a gender-dependent manner.

P7

ACCELERATION OF TUMOR METASTASIS BY ACTIVATED ERB THROUGH THE ENHANCEMENT OF MICROTUBULES DYNAMICS IN 4T1 BREAST TUMOR CELLS

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Tumor metastasis requires the inappropriate induction of cell migration. Cell migration needs the dynamic regulation of actin and microtubules. In MDA-MB-231 cells (a breast tumor cell line), activation of ERβ by estrogen enhances migration by extending actin-mediated lamellipodia. It is unknown whether activated ERβ increases cell migration by the enhancement of microtubules dynamics, thus the purpose of this study is to investigate if activated ERβ by estrogen accelerates lung metastasis of breast tumors by increasing tubulin-mediated cell migration. Subcutaneous implantation of 4T1 (an ERβ+ breast tumor cell line) induced tumor formation in BALB/C mice. Intraperitoneal injections of estrogen daily further increased tumor growth and lung metastasis, which was inhibited by PHTPP (an ERβ antagonist). Migration assay by using Boyden chambers showed PHTPP or ERβ sh-RNA inhibited the E2- and DPN- (an ERβ agonist)-increased migration in 4T1 cells. Disturbance of microtubule dynamics by paclitaxel and colchicine counteracted E2-increased migration. Estrogen induced the formation of long thick microtubule bundles scattered throughout the cytoplasm toward the membrane protruding regions as observed by using immunofluorescence analysis, which was reversed by PHTPP. Western blot analysis showed that a 3-hr treatment with E2 decreased the amount of α-tubulin but a 12-hr treatment did not. The decreased abundance of α-tubulin and acetylated α-tubulin by a 3-hr treatment with estrogen was reversed by PHTPP. Since activated ERβ transiently reduces the amount of α-tubulin, but does not affect the ratio of acetyl α-tubulin to α-tubulin. We hypothesize that activated ERβ increases breast tumor cell migration by enhancing microtubule dynamics, specifically accelerating the redistribution of tubulin to protruding regions which ultimately results in tumor growth and lung metastasis.

P8

MODULATION OF NUCLEOTIDE-EVOKED CALCIUM SIGNALLING BY THE NOVEL G-PROTEIN COUPLED RECEPTOR 30 (GPR30) IN HUMAN BRONCHIAL EPITHELIA

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Estrogen is an important hormone for both women and men, not only because of its capability to regulate a multitude of biological processes, but also because it is a significant target in many diseases, such as cancer and inflammation. In addition to the classical nuclear hormone estrogen receptors (ER) ERα and ERβ, a novel estrogen receptor, G-protein coupled receptor 30 (GPR30), was recently identified. Many of the rapid, non-genomic biological responses of estrogen are now attributed to an action on this novel membrane ER.

Our preliminary results have demonstrated that both primary normal human bronchial epithelial cells (NHBEs) and the 16HBE14o- cell line express the GPR30. We found that stimulation of these cells with the specific agonist of GPR30, G1, rapidly attenuated a UDP- and UTP-evoked increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). These results provide the first suggestion that GPR30 may function as a plasma membrane ER and interact with the P2Y receptor-mediated signaling pathway, which may affect the ion transport processes. In addition, we found the activation of the $P2Y_2$/$P2Y_4$ and $P2Y_6$ receptors with UTP and UDP, respectively, to stimulate a marked increase in interleukin (IL)-6 and IL-8 release by the airway epithelial cells, thus suggesting a pro-inflammatory role for the nucleotide receptors. Our data suggest that the activation of GPR30 may modulate the pro-inflammatory state of the airway epithelia by interfering with the P2Y receptor-mediated signaling pathway.

The work was supported by a Research Grant Council General Research Fund (#2140730) awarded to W. H. Ko.

P9

REGULATION OF IGF1 TRANSCRIPTION BY COMPLEX INTERACTION BETWEEN TRANSCRIPTIONAL FACTOR BINDING SITES AND MICROSATELLITE

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Microsatellite is a common type of short tandem repeat in the human genome, yet it is still not clear if they have any functional roles in the non-coding regions. In this study, we found differential transcriptional activities among common haplotypes of IGF1 promoter, which were accounted for by complex interactions, particularly interaction between microsatellite and SNPs. Also, for the first time, we demonstrated that a non-coding microsatellite polymorphism could act as a functional unit in human genome.

In the regulation of IGF1 expression, effect of the microsatellite was exerted by mediating the interaction between the upstream CCAAT/enhancer binding protein delta (CEBPD) transcription activation complex and downstream forkhead box A3 (FOXA3). A longer microsatellite was found to have a weaker transcriptional activity, suggesting a weaker interaction between two transcription complexes. Also, as CEBPD transcription complex binds exclusively to C allele at rs35767 (-1411C>T), it may account for the observation that such regulatory phenomenon due to microsatellite length was only presents in C allele but not T allele. Serial deletion assays showed that the regulation of haplotype with T allele may be accounted for by a 0.6kb fragment containing only the "C-A" portion. In conclusion, the microsatellite length in IGF1 promoter regulated transcriptional activity by mediating an interaction between two different transcription factor complexes located on its two sides.

P10

UPREGULATED TRPM2 AND ITS POTENTIAL ROLE IN NEOINTIMAL HYPERPLASIA

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A hallmark in atherosclerosis is progressive intimal thickening, which leads to occlusive vascular disease such as myocardial infarction and stroke. A causation of neointimal hyperplasia is the migration and proliferation of smooth muscle cells, in which reactive oxygen species (ROS) play an important role. This study was designed to investigate the involvement of TRPM2, a member of the transient receptor potential superfamily, in neointimal hyperplasia. Neointimal hyperplasia of rat femoral artery was induced by cuff placement. The TRPM2 expression and ROS generation were detected. The effect of TRPM2 inhibitors on neointimal hyperplasia of cultured human saphenous veins, and on proliferation and migration of primary rat aortic smooth muscle cells was further examined. It was found that arteries with cuff placement for 14 days showed distinct intimal thickening. The neointima area displayed an enhanced cell cycle activity and upregulated TRPM2 expression. ROS generation was dramatically increased in the neointima and media layer of arteries after cuff placement. After culture for 14 days, the human saphenous veins clearly showed neointimal hyperplasia, which was markedly reduced by *in vitro* treatment with TM2E3, a specific TRPM2-blocking antibody, or 2-aminoethoxydiphenyl borate, a chemical blocker. Both TM2E3 and 2-aminoethoxydiphenyl borate inhibited hydrogen peroxide-induced proliferation and wound-induced migration in primary rat aortic smooth muscle cells. These results suggest that TRPM2 is involved in neointimal hyperplasia and that blocking TRPM2 could be a way to prevent vascular wall thickening.

P11

CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR IS INVOLVED IN THE RELEASE OF ATP FROM CONTRACTING SKELETAL MUSCLE

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Contracting skeletal muscle releases ATP into the interstitial space where it is subsequently broken down to adenosine by the action of ecto-5'-nucleotidase. Both ATP and adenosine are vasodilators that contribute to the exercise hyperaemia. However, the mechanism for the release of ATP from muscle during exercise remains unknown. Cystic fibrosis transmembrane conductance regulator (CFTR) is involved in ATP release from muscle at low pH: this study was performed to investigate whether CFTR was involved in the ATP release from skeletal muscle during contractions.

Experiments were performed in rats anaesthetised with sodium pentobarbitone and breathing spontaneously. A microdialysis probe was placed in one gastrocnemius muscle: ATP was determined in interstitial microdialysate samples using a bioluminescence assay. The sciatic nerve was stimulated to induce two bouts of muscle contractions, separated by a recovery period of 40 mins; one of the inhibitors was administered prior to the second bout of contractions.

Muscle contractions elevated the interstitial ATP from 3.6 ± 0.4 to 80.8 ± 11.9 nM. In the control experiments, no drug was given: both the contractile force and the increase in interstitial ATP were reproducible in repeated contraction bouts. Infusion of a specific inhibitor of CFTR, $CFTR_{inh}$-172, did not alter the contractile force, but significantly lowered the interstitial ATP during muscle contractions, suggesting that CFTR is involved in the contraction-induced ATP release. Similarly, infusion of the Protein Kinase A inhibitor, KT5720, significantly reduced interstitial ATP during muscle contractions without altering contractile force, suggesting that CFTR in skeletal muscle is activated through the cAMP/PKA pathway. The increase in interstitial ATP during muscle contraction was also inhibited by the Na/H exchanger inhibitor, amiloride, or the Na/Ca exchanger inhibitor, SN6.

These data suggest that CFTR, activated through the cAMP/protein kinase A pathway, is involved in the ATP release during muscle contraction, and that activation of the Na/H exchanger and Na/Ca exchanger was also required, indicating that the signal transduction mechanism for CFTR activation during muscle contractions may be similar to that which is reported to occur at low pH.

P12

THE SIGNAL TRANSDUCTION PATHWAY FOR ACIDOSIS-INDUCED CFTR-MEDIATED ATP RELEASE FROM L6 SKELETAL MYOCYTES

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ATP is an extracellular signaling molecule involved in the regulation of skeletal muscle blood flow. Our previous study showed that depression of pH using lactic acid treatment stimulated the efflux of ATP from skeletal myocytes through a mechanism that involved the cystic fibrosis transmembrane conductance regulator (CFTR). The present study was undertaken to further explore the signal transduction mechanism linking the decrease in pH to the ATP release from muscle.

Accumulation of ATP in the medium surrounding the myocytes was measured using a luminescence assay. Incubation of the myocytes in lactic acid (10 mM) for 3 hours increased the extracellular ATP from 0.67 0.08 to 1.15 0.11 nM (n=36; P<0.001). Patch clamp studies showed that the whole-cell chloride current was increased at low pH, suggesting that a chloride channel may be involved in the ATP efflux. The specific inhibitor of CFTR, CFTRinh-172, abolished the increase in chloride conductance at low pH, and inhibited the lactic-acid-induced increase in extracellular ATP. Lactic acid treatment increased the intracellular cAMP from 3.2 0.3 to 7.1 1.0 nM, and the Protein Kinase A (PKA) activity from 30.6 4.5 to 37.0 5.4 pmol/ml, whereas the lactic-acid-induced increase in extracellular ATP was inhibited by the PKA inhibitor, KT5720, but enhanced by the phosphodiesterase inhibitor IBMX,

suggesting that the PKA/cAMP pathway was involved in CFTR activation. Amiloride, an inhibitor of the Na/H exchanger, prevented the lactic-acid-induced increases in intracellular cAMP and extracellular ATP; inhibitors of the Na/Ca exchanger, SN-6 and KB-R7943, also inhibited the lactic-acid-induced accumulation of ATP in the medium surrounding the cultured myocytes.

Based on these data, we propose that depression of the pH increases the activity of the Na/H exchanger, leading to increased intracellular Na: this drives reverse-mode operation of the Na/Ca exchanger, resulting in a localized increase of Ca in a microdomain close to the membrane, which then activates adenyl cyclase, elevating the intracellular cAMP; this, in turn, activates Protein Kinase A to phosphorylate CFTR, and CFTR finally regulates the opening of the ATP release channels.

P13

MOLECULAR MECHANISM OF CAPACITATIVE CALCIUM ENTRY DEFICITS IN FAMILIAL ALZHEIMER'S DISEASE

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Presenilin (PS) is the catalytic subunit of the gamma-secretase which is responsible for the cleavage of amyloid precursor protein to form beta amyloid (Aβ). Mutations in PS associated with familial Alzheimer's disease (FAD) increase the Aβ plaques formation in the brain and cause neurodegeneration. Apart from this, FAD-linked PS mutations have been demonstrated to disrupt intracellular calcium (Ca^{2+}) regulation. Accumulating evidence suggests that Ca^{2+} disruption may play a proximal role in the AD pathogenesis. Mutant PS exaggerated Ca^{2+} release from the endoplasmic reticulum (ER). It also attenuated Ca^{2+} entry through the capacitative Ca^{2+} entry (CCE) pathway, yet, the mechanism is not fully understood. Using a human neuroblast cell line SH-SY5Y and Ca^{2+} imaging technique, we observed CCE deficits in FAD-linked PS1-M146L retroviral infected cell. The attenuation of CCE in PS1 mutant cells was not mediated by the down-regulation of STIM1 and Orai1 expression, the known essential molecular players in the CCE pathway. Instead, we identified a molecular interaction between PS and STIM1 proteins by immunoprecipitation. On the other hand, immunofluorescence staining showed a significant reduction in puncta formation after ER Ca^{2+} depleted by thapsigargin in cells infected with PS1-M146L as compared to the wild type PS1 infected cells. Taken together, our results suggest a molecular mechanism for the CCE deficits in FAD associated with PS1 mutations. The interaction of mutant PS1 with STIM1 exerts a negative impact on its oligomerization and/or its interaction with Orai1. Our results may suggest molecular targets for the development of therapeutic agents that help to treat the disease.

P14

FUNCTIONAL TRANSIENT RECEPTOR POTENTIAL CHANNELS IN HUMAN CARDIAC C-KIT$^+$ CELLS

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Background and objective: Human adult c-kit$^+$ cardiac stem cell are characterized by the expression of c-kit in the absence of lineage markers such as Nkx2.5. They are self-renewing, clonogenic, and multipotent, giving rise to a minimum of three differentiated cell types: myocytes, smooth muscle, and endothelial vascular cells. These cells, although not specifically programmed for myocardial differentiation, have been shown to improve cardiac function in a myocardial injury/reconstitution assay. However, cell biology is not understood. The present study was to investigate the expression of transient receptor potential (TRP) channels in human cardiac c-kit$^+$ cells, and their role in regulating migration and proliferation.

Methods: Whole-cell patch voltage-clamp, RT-PCR, and Western blot approaches were used to determine functional expression of TRP channels in cultured human cardiac c-kit$^+$ cells. ShRNA targeting TRP channels were constructed to silence the related TRP channels. Wound healing and transwell

assay were applied to observe the effect of the TRP channels on cell migration. Cell proliferation assay was made with MTT and ^{3}H-thymidine incorporation approaches.

Results: A small background current was inhibited by the TRPC channel blocker La^{3+}. Removal of Mg^{2+} of pipette solution or bath solution induced a Mg^{2+}-sensitive TRPM7 current, and the current was suppressed by the TRP channel blocker 2-aminoethoxydiphenyl borate. RT-PCR revealed significant mRNA expression of TRPC1, TRPC3, TRPC4, TRPV2, TRPV4, and TRPM7 channels in human preadipocytes. Western blot analysis confirmed the protein expression of these TRP channels. ShRNAs targeting TRPV2, TRPV4 and TRPM7 suppressed the corresponding gene and protein expression. Interestingly, TRPV2-shRNA and TRPM7-shRNA significantly reduced proliferation of human cardiac c-kit$^+$ cells. Migration of human cardiac c-kit$^+$ cells was reduced by TRPV2-shRNA, TRPV4-shRNA.

Conclusion: Our results demonstrate for the first time that multiple TRP channels, TPC1/3/4, TRPV1/2/4, and TRPM7, are present in human cardiac c-kit$^+$ cells. TRPV2, TRPV4 and TRPM7 channels participate in regulating migration and proliferation in human cardiac c-kit$^+$ progenitor cells.

P15

PATCH-CLAMP STUDY OF SINGLE RYANODINE RECEPTOR CHANNELS IN THE OUTER NUCLEAR MEMBRANE

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Modulation of cytoplasmic free calcium (Ca^{2+}) concentration is a universal signaling pathway that regulates numerous cellular processes. Ubiquitous intracellular Ca^{2+} release channels – inositol 1,4,5-trisphosphate receptor ($InsP_3R$) and ryanodine receptor (RyR) channels – localized in the sarco/endoplasmic reticulum (ER) play a central role in this pathway in all animal cells. Electrophysiological study of the single-channel conductance and gating properties of these Ca2+ release channels with conventional patch-clamp approach has been hindered by their intracellular localization. To overcome this limitation, patch-clamp electrophysiology has been applied on isolated nuclei where these Ca^{2+} release channels are found abundantly in the outer nuclear envelope. We have successfully uterlized this nuclear membrane electrophysiology to study the gating properties of single $InsP_3R$ channels in several cellular systems. Whereas, all the current single channel data, including channel conductance, permeation properties, and ligand regulation, of the RyR channels were done exclusively by reconstituting the channels into artificial planar lipid bilayers. To gain insights into the single channel properties of the RyR in its native membrane milieu, we applied nuclear membrane electrophysiological study on isolated nuclei from stable-inducible mouse RyR2 HEK-293 cells. Using potassium as charge carrier, caffeine activated single channel current with conductance of ~750 pS in isolated nuclei. This caffeine activated current showed a linear current/voltage relationship under symmetrical ionic conditions and was sensitive to non-specific RyR inhibitor, ruthenium red. Furthermore, the single RyR channels recorded from the outer nuclear membrane exhibited bi-phasic Ca^{2+} regulation. In conclusion, we demonstrated, for the first time, that single RyR channels recordings from isolated nuclei and our results suggested that the nuclear membrane electrophysiology could be a sensitive and robust technique to study the gating properties of intracellular channels, including the $InsP_3R$ and RyR.

P16

ROLES OF FUNCTIONAL ION CHANNELS IN HUMAN CARDIAC C-KIT+ PROGENITOR CELLS

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Background and objectives: Cardiac progenitor cells play an important role in cardiac repair and regeneration; however, cellular biology and electrophysiology are not understood. The present study was to investigate the functional ion channel expression in human cardiac c-kit$^+$ progenitor cells and the

potential roles of these ion channels in regulating proliferation and migration.

Methods: Multiple experimental approaches were employed in this study, including whole-cell patch voltage-clamp, RT-PCR, Western blots, cell proliferation and migration assays, etc.

Results: Several ionic currents were heterogeneously expressed in human cardiac c-kit$^+$ progenitor cells, including a large conductance Ca^{2+}-activated K^+ current (BKCa) in most (93%) of cells, an inwardly-rectifying K^+ current (I_{Kir}) in 87% of cells, a transient outward K^+ current (I_{to}) in 39% of cells, a voltage-gated tetrodotoxin-sensitive Na^+ currents ($I_{Na,TTX}$) in 76% of cells. Molecular identities of these ionic currents were determined with RT-PCR and Western blot analysis. KCa.1.1 (for BKCa), Kir2.1 (for I_{Kir}), Kv4.2, Kv4.3 (for I_{to}), NaV1.2, NaV1.3, NaV1.6, NaV1.7 (for $I_{Na.TTX}$) were expressed in human cardiac progenitor cells. Inhibition of BK_{Ca} with paxilline, I_{to} with 4-aminopyridine, but not $I_{Na.TTX}$ with TTX and I_{Kir} with Ba^{2+}, decreased cell proliferation. Silencing of KCa.1.1, Kv4.2 or Kv4.3, but not Kir2.1, with siRNA targeting corresponding channels reduced proliferation. Inhibition of KCa1.1 or Kv4.2 or Kv4.3 channels accumulated cells at G0/G1 phase. Interestingly, down regulation of KCa1.1, Kv4.2 or Kv4.3 channels decreased, while of Kir2.1 channels increased migration in human c-kit$^+$ progenitor cells.

Conclusions: These results demonstrate for the first time that multiple ion channels are expressed in human cardiac c-kit$^+$ cells. KCa1.1, Kv4.2, and Kv4.3 channels, but not Na^+ channels and Kir 2.1 channels, participate in regulating proliferation. KCa1.1, Kv4.2 or Kv4.3 channels promote, while Kir2.1 channels reduce cell migration in human cardiac c-kit$^+$ progenitor cells.

P17

CHRONIC INTERMITTENT HYPOXIA INDUCES OXIDATIVE STRESS AND INFLAMMATION VIA ANGIOTENSIN II RECEPTOR 1 IN RAT LIVER

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Chronic intermittent hypoxia (IH) associated with obstructive sleep apnea (OSA) is characterized by repetitive cycles of hypoxia and reoxygenation, leading to excessive production of reactive oxygen species and oxidative stress in tissues and organs. However the mechanistic effects of chronic IH on the liver are not clear at present. We hypothesized that renin-angiotensin system (RAS) plays a role in the IH-induced oxidative stress and tissue inflammation in the rat liver.

Adult Sprague-Dawley rats were exposed to air (normoxic (Nx) control) or IH treatment (with inspired oxygen fraction in the normobaric chamber cyclic between 5-21% ± 0.5% per min, 8 hours per day) for 14 days. Rats were fed with an angiotensin II type 1 (AT1) receptors blocker telmisartan (10mg/kg body weight), or vehicle daily before the IH treatment. Hepatic expression levels of pro-inflammatory cytokines TNF-α, IL-6, and IL-1β were detected with ELISA assay; serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were examined for liver injury; also the level of oxidative stress with malondialdehyde (MDA) in the liver.

Our results showed that the protein expression of IL-6, TNF-α and IL-1β were significantly higher in the hypoxic group than that of the Nx control and telmisartan-treated hypoxic (TIH) groups, suggesting that inhibition of the binding of angiotensin II to AT1 receptors attenuates IH-induced tissue inflammation in the rat liver. In addition, the MDA level was significantly elevated in the hypoxic group but was normalized by the telmisartan treatment. Furthermore, the serum ALT to AST ratio was increased significantly in the hypoxic group when compared to the Nx and TIH groups.

In conclusion, blockade of the AT1 receptor mitigates oxidative stress, tissue inflammation and cellular injury in the liver of rats exposed to chronic IH mimicking a severe OSA condition, thus supporting a pathogenic role of RAS in the IH-induced hepatic injury.

P18

NADPH OXIDASE UPREGULATED BY AT1 RECEPTOR MEDIATES CHRONIC INTERMITTENT HYPOXIA-INDUCED OXIDATIVE STRESS AND INFLAMMATION IN RAT ADRENAL MEDULLA

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Our previous study found that chronic intermittent hypoxia (CIH) associated with recurrent apnea induced oxidative stress and inflammation in rat adrenal medulla. However, the underline mechanism was not clear. We hypothesized that, under CIH, the up-regulation of NADPH oxidase mediated by renin-angiotensin system (RAS) via an activation of angiotensin II receptor 1 (AT1) might take part in the oxidative stress and local inflammation in the adrenal medulla. Adult male SD rats were exposed to air (normoxic) control or CIH treatment (8 hours/day) which mimicked a severe recurrent sleep apneic condition for 14 days. Oral feeding of Telmisartan (10 mg/kg), a specific AT1 receptor blocker, or an intraperitoneal injection of apocynin (25 mg/kg i.p.), an inhibitor of NADPH oxidase, or vehicle was performed before the daily hypoxic treatment. The adrenal medulla was harvested for the measurement of markers for oxidative stress (MDA and NTR), macrophages infiltration (ED1), apoptosis, and inflammation (pro-inflammatory mediators) using TUNEL assay, real-time PCR, ELISA and Western blot. Levels of MDA and NTR were significantly increased in the hypoxic (CIH) group when compared with the normoxic control, but were normalized in the hypoxic groups treated with apocynin (AIH) or telmisartan (TIH). The expression levels of macrophage marker ED1-immunoreactivity and the pro-inflammatory mediators (TNFa, IL6) were also elevated in the CIH group, but were significantly ameliorated by the apocynin or telmisartan treatment. In addition, the amount of apoptotic cells in the CIH group was significantly higher than that of the AIH and TIH groups. Moreover, the mRNA levels of NADPH oxidase subunits (Nox2, Nox4) were increased significantly in the CIH group when compared with that of the AIH and TIH groups. Also, the protein expression of RAS components (AGT, AT1) was also increased in the CIH group. In conclusion, we showed that an up-regulation of NADPH oxidase via AT1 receptor activation mediates CIH-induced oxidative stress and inflammation in rat adrenal medulla.

P19

REDUCTION IN HEPATIC APOPTOSIS MODULATED BY GARLIC DERIVED S-ALLYLMERCAPTOCYSTEINE (SAMC) IN NON-ALCOHOLIC FATTY LIVER DISEASE RAT MODEL THROUGH P53-DEPENDENT PATHWAYS

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Purpose Previous study demonstrated that administration of garlic-derived antioxidant S-allylmercaptocysteine (SAMC) ameliorated hepatic injury in a non-alcoholic fatty liver disease (NAFLD) rat model. In the present study, we investigated the effect and mechanism of SAMC on NAFLD-induced cellular apoptosis in the liver.

Methods Adult Sprague-Dawley female rats were fed with a diet comprising of highly unsaturated fat diet (30% fish oil) for 8 weeks to develop NAFLD with or without intraperitoneal injection of 200 mg/kg SAMC three times per week. After chemical euthanasia, liver samples were collected for histological, biochemical and molecular analyses.

Results During NAFLD development, increased apoptotic cells were observed in the liver. Hepatic apoptosis was accompanied by activated intrinsic apoptotic pathway as shown by expressional changes of cytochrome c and Bcl-2 family genes. Extrinsic apoptotic pathway was also activated as shown by expressional changes of Fas, TRAIL, FADD and cleaved caspase-8. Increased activity of caspase-3 further confirmed the activation of apoptosis. In addition, reduced activity of LKB1/AMPK and PI3K/Akt pathways could be observed with increased expression of pro-apoptotic regulator p53 in NAFLD rats. Administration of SAMC reduced the number of apoptotic cells through down-regulation of both intrinsic and extrinsic apoptotic mechanisms. Phosphorylation status of LKB1, AMPK, PI3K, and Akt were also restored by SAMC co-treatment, leading to the reduction of p53 expression.

Conclusion Administration of SAMC during NAFLD development in rats protects liver from apoptosis through p53-dependent intrinsic and extrinsic apoptotic pathways.

P20

EPIDERMAL GROWTH FACTOR STIMULATES CELL PROLIFERATION BY ACTIVATING VOLTAGE-GATED POTASSIUM CHANNELS IN RAT BONE MARROW-DERIVED MESENCHYMAL STEM CELLS

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Background and objective: We have previously found that voltage-gated delayed rectifier potassium current (IK_{DR}, encoded by Kv1.2 and Kv2.1) participated in regulation of cell cycling progression in rat mesenchymal stem cells (MSCs) from bone marrow. The present study was designed to investigate whether epidermal growth factor (EGF) regulates cell growth is mediated by activating IK_{DR}.

Methods: Whole-cell patch voltage-clamp, RT-PCR, Western blots, siRNA, cell proliferation assay were employed in the present study

Results: EGF increased cell proliferation in a concentration-dependent manner, and the effect was countered by the broad spectrum protein tyrosine (PTK) inhibitor genistein and the EGFR kinase inhibitor AG556. We found that genistein and AG556 inhibited IK_{DR} in a concentration-dependent manner, The protein tyrosine phosphatase (PTP) inhibitor orthovanadate enhanced IK_{DR}, and counted the inhibitory effect of IK_{DR} by genistein or AG556, suggesting the PTK-mediating modulation of IK_{DR}. Interestingly EGF also increased IK_{DR}, Downregulation of IK_{DR} with siRNA targeting to Kv1.2 or Kv2.1 channels inhibited basal proliferation, and prevented EGF-stimulated proliferation in rat MSCs.

Conclusion: These results demonstrate for the first time that EGF stimulates cell proliferation activating IK_{DR}, and silencing Kv1.2 or Kv2.1 channels prevents the augmentation of proliferation by EFG, indicating that Kv1.2 and Kv2.1 channels mediate EGF effect in regulating cell growth in rat MSCs.

P21

THE NATURAL FLAVONE ACACETIN BLOCKS KV4.3 CURRENT BY INTERACTING WITH P-LOOP FILTER HELIX OF THE CHANNEL

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Background and objective: We have recently demonstrated that the natural flavone acacetin is an atrial-selective compound that inhibits ultra-rapid delayed rectifier potassium current (I_{Kur}) and transient outward potassium current (I_{to}) in human atrial myocytes, and also acetylcholine-activated potassium current ($I_{K.ACh}$). It increased atrial effective refractory period and effectively prevented atrial fibrillation (AF) in anesthetized dogs without prolonging QT interval of ECG. The present study was designed to investigate the potential molecular determinants of hK4.3 channels that encode human cardiac I_{to}.

Methods: Cell culture, mutagenesis and whole-cell patch voltage-clamp techniques were used in the present study.

Results: It was found acacetin inhibited hKv4.3 current in HEK 293 cells stably expressing Kv4.3 gene (*KCND3*) in a concentration-dependent manner. The current inhibition with an increase of time-to-peak and inactivation time constant of the current, suggesting an open channel blockade. However, the stimulation pause during drug administration revealed a strong tonic blocking property. This effect induced a use- or frequency-dependent inhibition at lower concentrations (1 and 3 μM), but not at high concentrations. The IC_{50} of acacetin for inhibiting hKv4.3 was reduced from 6.09 μM at 0.2 Hz to 5.80, 4.55, 3.96, and 3.65 μM respectively at 1, 2, 3, and 4 Hz. The mutagenesis study showed that the channel blockade by acacetin was dramatically reduced in hKv4.3 mutant T366A and T367A (IC_{50}, 197.8 μM for T366A and 166.1 μM for T367A) of the P-loop helix, and IC_{50} was also reduced in V392A, I395A, and V399A (IC50: 25.9 μM, 24.1 μM, and 9.5 μM) of the S6 domain.

Conclusion: These results demonstrate the novel information that acacetin is a tonic and open channel blocker of hKv4.3 by binding to T365 and T366 of the P-loop helix, and also interacts with V392, I395, and V399 of the S6 domain of hKv4.3 channels. The use- and rate-dependent blocking property of hKv4.3 by acacetin indicates that this natural compound could exert a strong suppressive effect in the treatment of tachycardiac arrhythmia diseases.

P22

HEPARANASE MEDIATES SYNDECAN-1 SHEDDING DURING CHRONIC AIRWAY INFLAMMATION

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Unopposed protease activity resulting from anti-elastase resistant supramolecular complexes of neutrophil elastase and shed syndecan-1 in the airway fluids is thought to be the cause of airway damage and deteriorating lung function in COPD and bronchiectasis (1, 2). We aim to develop strategies to decrease syndecan-1 shedding for the treatment of COPD and bronchiectasis.

Since sputum of such patients contain elevated levels of heparanse but not elevated levels of matrix metalloproteinase (MMP), we propose that the action of the GAG digesting enzyme heparanase on syndecan-1 modifies it such that it becomes more susceptible to shedding by MMP cleavage.

We show *in vitro* that heparanase induced syndecan-1 shedding in air-liquid interface cultured human epithelial cells, and that heparanase-treated recombinant syndecan-1 was more readily cleaved by MMP-7 than control treatment of the recombinant syndecan-1. Surface plasmon resonance analysis demonstrated an increase in affinity of syndecan-1 for MMP-7 upon heparanse treatment. Co-immunoprecipitation study targeting syndecan -1 found associated MMP-7, significantly higher in the sputum samples of bronchiectasis and COPD patients than in induced sputum of healthy individuals; normalized pixel density ratios of MMP-7: syndecan-1 reinforced the suggestion of increased affinity of MMP-7 for syndecan-1 that had been exposed to heparanase activity. The results support that heparanase activity in the inflammatory airway environment enhances shedding of syndecan-1 and thus a target for the treatment of bronchiectasis and COPD.

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P23

ADRENOMEDULLIN INHIBITS NOREPINEPHRINE-INDUCED CONTRACTION OF RAT SEMINAL VESICLE

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OBJECTIVE To investigate the effect of adrenomedullin (ADM) on seminal vesicle smooth muscle contractions in the rat and the specific receptor involved. Whether it was dependent on the nitric oxidant pathway was also investigated.

METHODS The seminal vesicles from Sprague-Dawley rats aged 8-10 weeks were incubated in Kreb's solution. Using an organ bath technique, the contraction of the seminal vesicle in response to norepinephrine (NE) and ADM was recorded, in the presence or absence of an ADM receptor blocker (hADM22-52), a calcitonin-gene-related peptide (CGRP) receptor blocker (hCGRP8-37), and L-NG-nitroarginine methyl ester, an endothelial nitric oxide synthase inhibitor. The basal tone, amplitude, and frequency of contraction were measured after incubation with the drugs.

RESULTS The results showed that the contraction induced by NE was effectively inhibited by ADM. The basal tone, amplitude, and frequency all decreased. The ADM effects on the NE-induced increases in basal tone and amplitude were completely blocked by

hCGRP8-37, the CGRP receptor antagonist, but were not abolished by L-NG-nitroarginine methyl ester.

CONCLUSION The findings have demonstrated that in the seminal vesicle the inhibitory effect of ADM on NE-induced contraction was mediated by the CGRP receptor but not by nitric oxide production.

P24

STABLE DERIVATION OF SCHWANN CELLS FROM HUMAN BONE MARROW STROMAL CELLS

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Background. Schwann cell transplantation improves post-traumatic nerve regeneration in both peripheral nervous system (PNS) and central nervous system (CNS) but sufficient numbers of immunocompatible cells are required for clinical application. Bone marrow stromal cells (BMSCs) are a readily accessible cell source that can be expanded and differentiated to specialized cells for regenerative medicine.

Objectives. We attempted to establish a protocol to induce the stable differentiation of human BMSC along a Schwann cell lineage.

Methods. Neurosphere medium was used to induce human BMSCs into neurospheres. Then, these neurospheres were induced to differentiate along the Schwann cell lineage using glia growth factors, and this was followed by co-culture with dorsal root ganglion (DRG) neurons.

Results. A lot of spheres of floating cells appeared after human BMSCs were cultured in neurosphere differentiation medium. These BMSCs-induced neuropheres showed nestin- and GFAP-immunoreactive staining. After induction with Schwann cell differentiation medium, a number of bi-polar and spindle-like cells positive for p75 and S100 grew from the neurospheres. However, these Schwann cell-like cells lost their Schwann cell phenotype after the induction medium was changed. Following co-culture with the DRG neurons, all derivatives of the Schwann cell-like cells not only acquired the Schwann cell phenotype, but remained stably committed even in subcultures in which both extrinsic factors and neurons were withdrawn.

Conclusion. Our success with stable derivation of Schwann cells from human BMSCs offer a viable source of Schwann cells for autologous cell therapy in clinical applications.

P25

INTERMEDIN IN REPRODUCTION: DISTRIBUTION IN MALE AND FEMALE REPRODUCTIVE SYSTEMS OF THE RAT; ITS CHANGES IN THE OESTROUS CYCLE; AND THE EFFECTS ON UTERINE CONTRACTION

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Intermedin (IMD), a peptide discovered in 2004, shared about 28% sequence identity with adrenomedullin (ADM)[1] and was later found to be identical to ADM2 discovered by another team[2]. Like ADM1, it acts on both CGRP (calcitonin-gene related peptide) and ADM receptors. A survey of the *Imd* mRNA levels in the male rat shows that the levels are lowest in the testis and the epididymis. The value in the seminal vesicle is the highest, followed by the coagulating gland and the ventral prostate. In the female the highest *Imd* RNA level is found in the oviduct, which is 10 times that of the ovary and 4 times that of the uterus. The *Imd* mRNA levels are low at dioestrus in the ovary and high at oestrus in the oviduct. The finding of *Imd* gene expression in the oviduct is similar to that of *Adm* in that for both mRNA levels, they are higher at oestrus[3]. In line with the findings for ADM[4,5], IMD inhibits the contraction of the uterus from immature rats injected with pregnant mare serum gonadotrophin to simulate oestrus. Both frequency and amplitude of contraction are decreased. These inhibitory effects are abolished by CGRP(8-37), the CGRP

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P26

THE HEDGEHOG SIGNALING IN MATURE OSTEOBLASTS AS A POTENTIAL REGULATOR FOR GLUCOSE HOMEOSTASIS AND PANCREATIC ISLET FUNCTION

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The pathogenesis of type 2 diabetes (T2DM) is characterized by pancreatic islet dysfunction followed by insulin resistance, thus leading to impaired glucose homeostasis and chronic complications. As a well-known regulatory factor for mammalian cell development, Hedgehog (Hh) signaling has been recently reported as a key modulator of bone formation and resorption in the skeleton which are related to the development of osteoporosis. Meanwhile, it has also been shown that insulin signaling in the skeleton regulates whole body glucose homeostasis via mediation of osteoblast development. As such, a potential skeleton-endocrine axis may exist that plays a role in islet cell function and T2DM. We thus hypothesize that regulation of Hh signaling in mature osteoblasts contributes to aberrant glucose homeostasis and islet cell function. To test this hypothesis, we employed a genetic engineered *Patched (Ptch1)* mutant mouse with an ubiquitous activated Hh signaling in mature osteoblasts and *Smothened*(*Smo*) mutant mouse with a decreased mature osteoblasts Hh signaling. The activated Hh signaling in mature osteoblasts and abnormal bone turnover in *Ptch1* mutant mice showed a decreased body weight, blood glucose, altered islet insulin/glucose distribution, marginal changes in glucose tolerance, insulin secretion and several hepatic glucose homeostasis related genes expression. On the other hand, inhibition of the Hh signaling in mature osteoblasts exhibited no effect on blood glucose and glucose tolerance, indicating that the mature osteoblasts might be involved in the regulation of glycemic control. Although the target linkage among the bone Hh, pancreatic islets and glycemic control is far from full elucidation, the present study provides a potential mechanism whereby the islet cell function is related to bone function which has clinical insight into T2DM and/or osteoporosis related islet dysfunction.

P27

REGULATION OF HYPOXIA-INDUCIBLE FACTOR 1 α (HIF-1 α) AFTER TRAUMATIC BRAIN INJURY

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Traumatic brain injury (TBI) is one of the most prevalent causes of morbidity and mortality all over the world. It affects young adults mainly in their most productive stage of life which produce long lasting disability in 25% of cases and make an enormous social and economical cost. TBI induced intracranial hemorrhage and brain edema which resulted in hypoxia and ischemia. Our previous studies suggested that the vascular endothelial growth factors (VEGF) mediated the TBI-induced hippocampal neurogenesis though MAPK pathway. Hypoxia inducible factors (HIFs) are normally scarce in cells, but greatly up-regulated during some pathological events such as ischemia and hypoxia. HIFs can serve as a transcription factor and regulate the expression of several genes which are responsible for angiogenesis, anaerobic oxidation, vasodilation and erythropoiesis. Our present studies found that the expression of HIF-1α and VEGF was significantly up-regulated in hippocampus beginning eight hours after TBI. Administration of HIF-1α inhibitor, 2-methoxyestradiol (2ME2, I.P), effectively reversed the TBI-induced hippocampal HIF-1α and VEGF upregulation. HIF has three isoforms including HIF1α, HIF2α and HIF3α.

In the present study, we found HIF1α is critical to VEGF regulation after TBI, but not HIF2α and HIF3α. The increase of HIF-1α in hippocampus not resulted from the HIF-1 gene activation but from the decline in HIF-1 degradation which regulated by PHD family.

P28

DOMINANT ROLE OF HIF-1a IN THE SUSCEPTIBILITY OF NEURONS AND GLIA DURING HYPOXIA

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As its key components, neurons and glia are intimately associated with the physiological and pathological processes in the brain. At the same time, hypoxia in the central nervous system is a commonly mentioned phenomenon under clinical conditions that include brain stem death, respiratory failure, organophosphate poisoning, ischemia and stroke. It follows that the susceptibility of neurons and glia in brain to hypoxia may determine the final fate of individuals against this insult. The present study aimed to compare the susceptibility of neurons and glia to the same hypoxic condition, and delineate the underlying mechanisms, using neuronal cath.a cell line and astroglial C6 cell line. Compared to normoxic condition, cath.a cells incubated under hypoxia (1% oxygen) for 1, 4, 6, 12 or 24 h underwent a time-dependent increase in cell viability as determined by the XTT method, without significant changes in the level of activated caspase 3. On the other hand, C6 cells exhibited a time-dependent decrease in cell viability, accompanying by apoptosis as indicated by augmented activated caspase 3. At the same time, the mRNA level of hypoxia inducible factor-1α (HIF-1α) was decreased in cath.a cells, but was increased in C6 cells, again in a time-dependent manner after hypoxia. Furthermore, the viability of both cath.a cells and C6 cells under normoxic condition was dose-dependently decreased on application of chemical inducers of HIF-1α (0-800 μM), cobalt chloride or desferrioxamine. We concluded that the level of HIF-1α plays a dominant role in determining the susceptibility of neuronal cath.a cells and astroglia C6 cells under hypoxic condition.

P29

ROLE OF INTRACELLUALR pH REGULATION MECHANISMS IM RETINOIC ACID-INDUCED P19 NEURON DIFFERENTIATION

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Intracellular pH (pH_i) is an important parameter in cell physiology. Transporters directly involve in the movement of base and acid across the cell membrane are classified as acid loaders (including Cl^- / HCO^-_3 transporters) or acid extruders (including Na^+/H^+ exchanger) depending on the direction of movement of base and acid. pH_i influences many processes important for cell growth and differentiation. We studied the time course and intracellular distribution of protein expression of AE3 isoform of Cl^-/HCO_3^- exchanger and NHE-1 isoform of Na^+/H^+ exchanger in retinoic acid (RA)–induced P19 cell differentiation. Recent experimental results from our lab prove that, AE3 is essential for neuronal differentiation because it is responsible for the accumulation of Cl^- in the cell. Our results show that after RA treatment, the total protein level of AE3 remain the same. However in untreated P19 cells, AE3 is mainly located in P19 cell nucleus. In response to RA treatment, AE3 is translocated to plasma membrane on P19 neuron so its ability to mediate Cl^--HCO_3^- exchange becomes more efficient. In addition, we also detected an increased gene expression of NHE-1 during P19 cell differentiation. The overall protein level of NHE-1 is increased during early stage, both in cytosol and plasma membrane. In later stage of RA-induced P19 cell differentiation, NHE-1 is mainly expressed in glial cells. We propose that difference in expression and distribution of AE3 and NHE-1 may influence P19 neuron and glial cell differentiation. In the later stage, NHE-1 in glial cells may supply acid for AE3 in P19 neurons to promote its neuron differentiation.

P30

TRKB/ERK SIGNAL IS INVOLVED IN EXERCISE-INDUCED THE PREVENTION OF NRF2 DOWNREGULATION AND NEURON LOSS AFTER MPP^+ TREATMENT

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Parkinson's disease (PD) is a progressive loss of dopamine (DA) neurons in the substantia nigra and results in movement disorder. Our preliminary results showed that treadmill exercise prevented the 1-methyl-4-phenylpyridinium (MPP^+)-induced nigrostriatal DA neurodegeneration and Nrf2 (nuclear factor erythroid 2-related factor 2, a key transcription factor in the regulation of antioxidant enzymes) inactivation. Other reports also indicated that exercise training induced a transient increase in brain-derived neurotrophic factor (BDNF) level and sustained upregulation of TrkB (a receptor of BDNF) protein expression. The downstream PI3K/AKT and MAPK/ERK pathway of TrkB receptor have been shown to be involved in Nrf2 activation. Therefore, we hypothesized that treadmill exercise upregulates TrkB-AKT/ERK pathway to prevent MPP^+-induced Nrf2 downregulation and DA neuron loss in rat nigrostriatal dopaminergic system. We found that the protein expression of total TrkB, phosphorylated TrkB (p-TrkB), and nuclear Nrf2 was significantly decreased at 24 and 72 h after MPP^+ treatment. Treadmill exercise for 4 weeks significantly increased the protein expression of p-ERK and prevented the MPP^+-induced downregulation of tyrosine hydroxylase (TH, a marker enzyme of dopamine neurons), p-TrkB, TrkB and nuclear Nrf2 protein expression in striatum 72 h after MPP^+ treatment. To identify the essential role of TrkB, we treated rats with TrkB inhibitor K252a or lentivirus-carried shTrkB and found that TrkB inhibition significantly attenuated exercise-induced the prevention of TH, p-TrkB, ERK and nuclear Nrf2 protein downregulation and enhanced MPP^+-induced neurotoxicity. The results demonstrated that treadmill exercise may regulate TrkB/ERK signaling pathway to prevent MPP^+-induced Nrf2 downregulation and neurotoxicity.

P31

CDK4 AND CDK5 INHIBITION IMITATES COLD EXPOSURE IN THE PREVENTION OF MITOCHONDRIAL FISSION AND NEURON DEATH AFTER MPP^+ TREATMENT

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Mitochondrial dysfunction is an early event of cell death in neurodegenerative diseases, such as Parkinson's disease. Mitochondrial dynamics of fusion and fission, is respectively controlled by mitofusion and dynamin-related GTPase (Drp1). Our preliminary results showed that a Drp1-dependent mitochondrial fission and neuron death was found in a 1-methyl-4-phenylpyridinium (MPP^+)-induced parkinsonian model. We also found that cells cultured in 32°C (mild cold exposure) significantly reduced MPP^+-induced cell death and prolonged cell cycle, which is associated with a decreased expression of cyclin-dependent kinases (CDKs). Recent study demonstrated that mitochondrial dynamics change at different stage of cell cycle and CDK5 is involved in the regulation of mitochondrial fission during neuron apoptosis. Herein, we investigated whether cold exposure protects neurons from MPP^+ intoxication by reducing the Drp1-dependent mitochondrial fission and modulating the expression of CDKs. By using the mitoDsRed-labeled human SK-N-SH cells, we observed that cold exposure for 24 h after MPP^+ treatment significantly reduced MPP^+-induced mitochondrial fission. We also found that the inhibition of CDK4 and CDK5 imitated the effect of cold exposure to inhibit MPP^+-induced mitochondrial fission and neuron death. Furthermore, results from Western blot revealed that cold exposure and inhibition of CDKs reduced Drp1 translocation to mitochondria. The effect of cold exposure and CDK inhibition in the prevention of mitochondrial fission was also found in rat primary cortical neurons. The results suggested that the inhibition of CDK4 and CDK5 may involve in cold exposure-induced neuroprotection by reducing Drp1-associated mitochondrial fission after MPP^+ treatment.

P32

THE NOVEL GENES *FAM134C* AND *C3ORF10* ARE REGULATED BY NRF-1 AND FUNCTION IN NEURITE OUTGROWTH

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Neurite outgrowth is an important process in neuronal development. Nuclear respiratory factor-1 (NRF-1) is a major transcription factor in the human genome and plays a key role in neurite outgrowth. We used bioinformatic tools to search for novel genes downstream of NRF-1 that mediate this function. *C3orf10, FAM134C*, and *ENOX1* were investigated because they are expressed in the nervous system and their NRF-1 response elements are conserved in humans and mice. We found that NRF-1 positively regulated *FAM134C* and *ENOX1*, but negatively regulated *C3orf10* in human neuroblastoma IMR-32 cells and primary cortical neurons. In IMR-32 cells, *FAM134C* positively regulated and *C3orf10* negatively regulated neurite length and induction, but *ENOX1* did not affect neurite outgrowth. *FAM134C* but not *C3orf10* shRNA regulates NRF-1-enhanced neurite outgrowth. Predicted structure and confocal images suggested that FAM134C is localized in the plasma membrane and cytosolic organelles, and C3orf10 is localized in cytosol and nucleus. Expression profiles of rat *Nrf-1*, *Fam134c* and *C3orf10* in cultured hippocampal neurons and brain tissues at different developmental stages suggest their roles in neuronal differentiation. In rat hippocampal neurons, *Fam134c* regulates neurite elongation and *C3orf10* regulates neurite branching. Overall, we annotated *FAM134C*, *C3orf10*, and *ENOX1* as NRF-1-regulated genes which have differential effects on neurite outgrowth.

P33

TGF-B SIGNALING IS INVOLVED IN LEIOMYOMAL CELL AGGREGATION IN A LONG-TERM CULTURE MODEL

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Leiomyoma, a benign tumor derived from myometrium, is the most common gynecological problem to cause hysterectomy. Previous studies have shown that leiomyoma expresses higher levels of TGF-beta and extracellular matrix compared to myometrium. Most studies on leiomyomal cells performed short-term culture. However, it was reported that leiomyomal cells placed in dish form ball-like cell aggregation after long-term culture. The mechanisms responsible for leiomyoma formation remain poorly understood. This study was aimed to investigate the role of TGF-β in leiomyomal cell aggregation in long-term culture. Leiomyomal cells were obtained from patients who underwent hysterectomy. Cells were cultured in DMEM containing 0.5% or 2% fetal bovine serum for seven or fourteen days. The cell aggregates were classified according to cell density detected by the phase-contrast photographs and Hoechst 33342 staining. Type A aggregates exhibited lower cell density whereas type B aggregates had higher density, a feature mimicking spheroid. In cells cultured under both conditions, TGF-β exhibited the trend to increase type-B aggregates but did not significantly affect type-A aggregates. SB431542, an inhibitor for TGF-β receptor kinase, inhibited TGF-β-stimulated cell aggregation in leiomyomal cells from four out of five patients. Phosphorylation of smad-3 was stimulated by TGF-β in a time-dependent manner, exhibiting peak activation at one hour. SB431542 eliminated TGF-β-induced smad-3 phosphorylation. These results suggest that TGF-β signaling is involved in leiomyomal cell aggregation.

P34

STRESS IN ADOLESCENT RATS INDUCES FEAR MEMORY DEFICITS VIA REDUCING NEUROPLASTICITY IN THE AMYGDALA

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Stress induces long-term adaptations in various brain regions, particularly the amygdala which mediates the stress responses and governs the fear learning and memory. In adult rodents, chronic stress not only improves amygdala-related fear learning and memory but also enriches dendritic arborization in basal and lateral nuclei of the amygdala. However, since the adolescence is a critical period for post-natal

developmental changes in the brain, adolescents may be more vulnerable than adults to the influences of chronic stressors. In this study we characterized the various stress-induced changes in adolescent rats. Three-wk-old male Sprague Dawley rats were divided into control and stress groups, the latter was subjected to 5 weeks of social instability stress consisting of daily cage change, roommate change and 1-hr immobilization. Our results indicated that chronic social instability stress in adolescent rats 1) reduced weight gain and food intake; 2) impaired the performance of fear-potentiated startle (an amygdala-dependent learning and memory task); 3) down-regulated amygdalar neuroplasticity-related proteins, including TrkB, synaptotagmin I and SNAP-25; 4) suppressed dendritic arborization of neurons in basal and lateral amygdala. Taken together, our results showed that chronic social instability in adolescence suppressed the amygdala-dependent learning and memory, and reduced neuroplasticity proteins and dendritic branches in the amygdala. Therefore, stressor exposures at different stages of life appear to differentially affect the amygdalar structure and function.

P35

ACOUSTIC REFLEXES: POTENTIAL USE IN PROBING TINNITUS IN ANIMAL MODELS

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In estimate the presence of tinnitus in animals, polydipsia avoidance (a learned behavior) and silence gap suppression of the acoustic startle reflex (pre-pulse inhibition) are commonly used. Though the polydipsia avoidance is more accurate, it requires long period of training. Reflexes are more straightforward and time-efficient (as no behavioral training is needed), but the acoustic startle reflex occurs at high sound intensity levels (>90 dB SPL), way above the perceptual level of tinnitus (<35 dB SPL). Hence, the use of other kinds of acoustic reflexes that can be elicited at low intensities would be very beneficial. However, the characterization of other acoustic reflexes (e.g., head orienting reflex, pinna reflex, freezing reflex) has not been done especially at low intensity levels when tinnitus is normally perceived. To study these three acoustic reflexes, we here developed an imaging system that is non-invasive. To minimize habituation, we presented a host of acoustic signals (including a set of pre-recorded environmental sounds with broad spectra). In adult rats, we observed (a) head orienting reflex was often observed at a longer latency (>100 ms); (b) concurrent with this, pinna movements that are ipsilateral to sound source with response latency <50 ms; (c) vibrissa freezing response that appeared with the shortest latency (25-50 ms). The different response latencies are consistent with the different neural pathways each response is mediated through. Most importantly, all these three reflexes could be induced by sounds at levels below 35 dB SPL. Results strongly suggested that these reflexes could be useful as a simple and objective means of probing the tinnitus through masking effect. (*Supported by National Science Council, Taiwan.)*

P36

EARLY SOUND EXPOSURE ENLARGED NEURONS IN THE AUDITORY DESCENDING SYSTEM OF JUVENILE RATS

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Early sound exposure increases neuronal size in rats during post-natal week-4. Changes appear in the primary auditory cortex and midbrain (or specifically, the central nucleus of the inferior colliculus). We speculate, based on the frequency-biased changes especially in the cortex (i.e., towards high- rather than low- frequency region of the exposing tone) that the descending auditory system could be involved. To test this hypothesis, we measured neuronal sizes in two centers of the auditory descending system, viz., the dorsal nucleus of the inferior colliculus (DIC) and the lateral nucleus of the superior olivary complex (LSO) with or without sound exposure during postnatal week-4. Starting from postnatal day-22, young rats were exposed to a low-frequency tone (4 kHz, 65 dB SPL) for a period of 7 days before sacrifice. Neurons were analyzed morphometrically from 7 um-thick histological sections. After sound exposure, both nuclei and perikarya expanded markedly in DIC (~55%) and less so in the LSO (~35%) ($p<0.0001$, Student's t-test). No similar changes were detected in the ascending auditory pathways of the lower brainstem (e.g., cochlear nucleus) or in the visual pathways (superior colliculus, visual cortex). Results strongly suggested

that sound-induced changes observed previously in the auditory cortex and midbrain involved the corticofugal pathways, and that these animals likely experience tinnitus which is biased towards high frequencies. *(Supported by National Science Council, Taiwan.)*

P37

PHYSIOLOGICAL ROLES OF CA^{2+}-ACTIVATED CHLORIDE CHANNELS IN DORSAL ROOT GANGLION NEURONS

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In sensory neurons, the Ca^{2+}-activated chloride channel (CaCC) plays a critical role in decoding sensory stimuli. One important feature of CaCC is that its Cl^- current is driven by the elevation of the cytosolic Ca^{2+}concentration. Previous studies have reported that CaCCs are present in dorsal root ganglion (DRG) neurons, but the exact function in DRG neurons is unknown. For example, it is unclear whether the CaCC current is one of components in the current induced by capsaicin, a compound that opens the Ca^{2+}-permeable cation channel [transient receptor potential vanilloid 1 (TRPV1)]. Our recent studies have further indicated that ~20% of the recorded DRG neurons contained both the CaCC current and the capsaicin-induced current. We hypothesize that the opening of TRPV1 channels may cause an increase of intracellular Ca^{2+} concentration, leading to a further activation of the CaCC current that could modulate the capsaicin-induced current. In the present study, we initially selected four subtypes of CaCC protein antibodies (Bestrophin, TTYH3, TMEM16a, and TMEM16b) to detect protein levels in acutely dissociated rat DRG neurons by use of Western blotting. Our results revealed that all four subtypes of CaCC proteins were expressed in DRG neurons. Among them, TMEM16a and TTYH3 were two major proteins. Moreover, immunofluorescence of rat DRG neurons revealed that all three subtypes of CaCC proteins (TMEM16a, Bestrophin, and TMEM16b) were co-localized with TRPV1 in naïve DRG neurons. About 35% of TRPV1-positive DRG neurons expressed CaCC proteins. In addition, the co-localized neurons almost were medium-size (30-40 μm) neurons. Furthermore, CaCC blockade reduces capsaicin-induced nocifensive responses. Capsaicin receptor activation has been implicated in nociception and inflammatory thermal hyperalgesia. The proposed studies will provide a new understanding of how CaCCs interact with capsaicin receptors and this knowledge may offer new strategies to reduce the pain.

P38

INVOLVEMENT OF *NUCLEAR RESPIRATORY FACTOR-1* AND *INTEGRIN ASSOCIATED PROTEIN* IN PROLIFERATION OF HUMAN MEDULLOBLASTOMA CELLS

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Brain development and tumorigenesis are highly related. Medulloblastoma is the most common malignant brain tumor in children. It is thought to result from the transformation of granule cell precursors (GCP) in the developing cerebellum. Studies showed that *integrin-associated protein* (*IAP*) is one of the genes down-regulated in mouse medulloblastoma, as compared to GCP. However, the role of *IAP* in medulloblastoma remains unknown. Our recent findings revealed that the expression of *IAP* is up-regulated in post-mitotic granule neurons in developing mouse cerebellum. We also found that *IAP* is critically regulated by the transcription factor nuclear respiratory factor 1 (NRF-1) which promotes neuronal differentiation in human neuroblastoma cells. Therefore, we hypothesized that *NRF-1* and *IAP* play roles in cell proliferation in medulloblastoma. We used the human medulloblastoma Daoy cells for investigation. Expressions of *NRF-1* and *IAP* gene in Daoy cells were confirmed by RT-PCR analysis. Overexpression of *NRF-1* or *IAP* decreased Brdu-positive cells while knockdown of *NRF-1* or *IAP* increased Brdu-positive cells, suggesting that *IAP* and *NRF-1* involve in cell proliferation in Daoy cells. Colony formation assays showed that knockdown of *NRF-1* and *IAP* increases the number of colonies. However, treatment with IAP agonist peptide 4N1K significantly decreased the number of colonies in a dose dependent manner. The MAPK inhibitor U0126 reversed the inhibition of cell proliferation by overexpression of *NRF-1* and *IAP*. Knockdown of *IAP* up-regulated the expression of cell cycle regulator cyclin D1. Our findings indicated that *NRF-1* and *IAP* inhibit proliferation of medulloblastoma cells via

the MAPK pathway and cell cycle regulator cyclin D1. Our results also suggest that activation of IAP is a potential therapeutic target for the treatment of medulloblastoma.

P39

MECHANISMS UNDERLYING EXERCISE TRAINING-INDUCED ENHANCEMENT OF LONG-TERM POTENTIATION IN THE RAT LIMBIC SYSTEM

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Several studies have indicated that chronic exercise training facilitates learning and memory as well as long-term potentiation (LTP), the best-described neurobiological substrate of learning and memory to date. Previous studies have suggested that serotonin (5-HT) plays a suppressing role in fear memory formation through activating 5-HT_{1A} receptors. A recent study further shows that four-week treadmill exercise enhances passive avoidance (PA) memory in rats by down-regulating 5-HT_{1A} receptor activation in the amygdala and hippocampus, brain areas highly associated with PA memory. However, whether chronic exercise training can improve LTP in the amygdala and hippocampus through attenuation of 5-HT_{1A} receptor activation is still unknown. In the present study, we used the extracellular electrophysiological recording technique and pharmacological methods to characterize the effects of four-week treadmill exercise on high-frequency stimulation (HFS)-induced LTP in the rat lateral amygdala (LA) and hippocampal dentate gyrus (DG), as well as the roles that the 5-HT_{1A} receptor and its downstream signals play in these effects. Our results revealed that four-week treadmill exercise enhanced both HFS-induced LA- and DG-LTP in male rats. Bath perfusion of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, abolished exercise-induced enhancement of LA- and DG-LTP. Interestingly, the PKA inhibitor H-89 only blocked the enhancing effect of exercise on DG-LTP but not LA-LTP. Moreover, nifedipine, an L-type voltage-gated calcium channel blocker, abolished the facilitating effect of exercise on both LA- and DG-LTP. In summary, the present data demonstrate that exercise training promotes LA- and DG-LTP, and the promoting effect of exercise training on LTP in these two brain regions may act through attenuating the activation of 5-HT_{1A} receptors and affecting distinct downstream signal pathways.

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NEONATAL DEXAMETHASONE TREATMENT INDUCE DEPRESSION-LIKE BEHAVIOR IN ADULT RAT

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Numerous literatures have indicated that stress and medication experience in early postnatal would generate subtle modulation in brain maturation, which in turn will result in long-lasting behavioral changes when animals were exposed to stress in the later period of life. Synthetic glucocorticoid, dexamethasone (DEX), is frequently used to ease the progression of chronic lung disease in premature infants. Recent studies suggest that neonatal DEX treatment impair brain development and cognitive functions. In the present study, forced swimming test (FST) was came out to evaluate the effect of neonatal DEX treatment on amygdale function in adulthood. Rats were subjected to receive subcutaneous injection of tapering doses of DEX (0.5 mg/kg, 0.3 mg/kg and 0.1mg/kg) from postnatal day 1 to 3 (PN1~PN3). Behavior test was performed at the age of 8 weeks. Since previous studies had shown that the MAPK signal pathway in amygdale played an important role in the manipulation of fear memory formation, the possible role of amygdale MAPK activation in the immobility behavior during FST was also investigated. Our results demonstrated that the immobility time was significantly increased in neonatal DEX treated rats. Intra-cerebroventricular infusion of MAPK inhibitor PD98059 suppressed immobility time of neonatal DEX treated rats during FST. These results suggested that DEX treatment during the neonatal period might induce a long-lasting increase in depression-like behaviors, and the amygdale MAPK activation might be involved in the formation of depression-like behaviors in neonatal DEX treated animals.

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EFFECTS OF CHRONIC FLUOXETINE TREATMENT ON HIPPOCAMPAL NEUROGENESIS IN THYROIDOCOMIZED RATS

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Thyroid hormones (TH), thyroxine (T4) and triiodothyronine (T3), produced in thyroid gland, were known to play important roles throughout prenatal and postnatal nervous system development. In rodents, T3 is required for neurogenesis in hippocampus. Additionally, disturbances of thyroid function or the adult neurogenesis might significantly affect mental status, such as depression. Recently, the thyroidectomy including partially or entirely *removal of the thyroid gland has been widely used as an animal* model for studying depression-like behavior.

Fluoxetine, a selective serotonin reuptake inhibitor (SSRIs), is commonly used for *treating depression. T*he present study was aimed *to investigate the effect of fluoxetine on* neurogenesis and depression-like behavior in thyroidectomy model. We found the thyroidecomized rats showed depression-like response in force swimming test and exhibited a significant decline in the number of immature neurons in the dentate gyrus of the hippocampus. Chronic fluoxetine treatment (by oral, 10 mg/kg) enhanced depression-like response in thyroidocomized animals. However, the neurogenesis in the dentate gyrus of thyroidecomized rats could not be recovered by chronic fluoxetine treatments. Our studies suggested that the thyroid hormone may participate in the therapeutic effect of fluoxetine.

P42

FUNCTIONAL ROLE OF TRPC5 CHANNELS IN AORTIC BARORECEPTOR

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TRP channels are a superfamily of non-selective cation channels that can be divided into seven subfamilies: TRPA, TRPC, TRPM, TRPML, TRPN, TRPP, and TRPV. Many TRP isoforms have been reported to be sensors for diverse source of external and/or internal stimuli. Recently, one of the isoforms, TRPC5, has been reported to be hypo-osmolarity and pressure sensitive.

Aortic baroreceptor is the mechanosensor to detect blood pressure in aortic arch. Upon changes in arterial blood pressure, the baroreceptive nerve terminal on the aortic arch adventitia will be activated, resulting in action potentials that propagate to the cardiovascular control centre in the brain. However, the molecular identity of the baroreceptor mechanosensors is not well understood.

In the present study, immunohistochemistry demonstrated the expression of TRPC5 channels in the aortic baroreceptor nerve terminal, which is located on the aortic arch, along the nerve fiber (aortic depressor nerve) and in the ganglion region (nodose ganglion). RT-PCR and immunoblot studies confirmed the expression of TRPC5 channels in the aortic baroreceptor. In Ca^{2+} imaging studies of cultured aortic baroreceptor neurons, a TRPC5 potentiator daidzein was able to potentiate the hypotonicity-induced $[Ca^{2+}]_i$ response while a TRPC5 blocking antibodies T5E3 inhibited the response. Electrophysiological studies showed that hydrostatic pressure could activate the whole-cell current in cultured baroreceptor neurons and the current displayed a double rectifying *I-V* relationship, which is typical of TRPC5. Daidzein treatment also potentiated the pressure-induced action potential firing in isolated aortic baroreceptor neurons, which could be blocked by a TRPC blocker 2-APB. Furthermore, *trpc5* knockout mice manifested a significant reduction in aortic depression nerve activity upon blood pressure elevation when compared with wild-type mice.

Taken together, our study provides the evidence that TRPC5 is involved in pressure sensing of aortic baroreceptor neuron and is participated in the aortic baroreceptor function.

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NEUROPROTECTIVE EFFECTS OF LYCIUM BARBARUM POLYSACCHARIDES AGAINST RAT HIPPOCAMPAL APOPTOSIS INDUCED BY CHRONIC INTERMITTENT HYPOXIA

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We have shown neuronal apoptosis in the hippocampus of rats exposed to chronic intermittent hypoxia mimicking severe conditions of obstructive sleep apnea (OSA) syndrome in patients [1]. Lycium barbarum polysaccharides (LBP), active biological ingredients of traditional Chinese herbal medicine Goji, have been shown to possess cytoprotective properties [2].

The aim of this study was to examine the protective effects of LBP against neuronal apoptosis in the hippocampus in a severe OSA rodent model. We hypothesized that oral administration of LBP ameliorates neuronal apoptosis in the rat hippocampus induced by chronic intermittent hypoxia.

Adult SD rats were randomly divided into 4 experimental groups, namely: (i) normoxic control (Nx); (ii) Nx treated with LBP; hypoxic groups treated with either (iii) LBP or (iv) vehicle. The hypoxic groups were kept in a normobaric chamber with inspired oxygen alternating from 21 to 5 ± 0.5% oxygen per minute for 8 hr/day for 7 days, whereas Nx groups was maintained in room air for 7 days. LBP (1mg/kg) were orally fed to the rats 2 hours prior the daily hypoxic treatment. Rats were sacrificed and the hippocampus was harvested for measurements of oxidative marker, malondialdehyde (MDA), apoptotic cell death using TUNEL assay, protein expression levels of antioxidant enzymes, and inflammatory cytokines by Western blot.

There were significantly more TUNEL positive –labeling cells in the CA regions and dentate gyrus of the hippocampus in the vehicle-treated hypoxic group than those of the Nx control and LBP-treated groups. In addition, levels of MDA and the protein expressions of cleaved caspase 3 and inflammatory cytokines were increased in the vehicle-treated hypoxic group when compared to the Nx groups and were lowered by the LBP treatment. Intriguingly, there were significantly more PCNA-labeling cells in the dentate gyrus of the hippocampus in the LBP-treated hypoxic groups than those of the other groups. Also, the protein expression of cyclin D1 was increased in the hypoxic groups when compared to the Nx groups.

In conclusion, oral administration of LBP significantly ameliorates oxidative stress, inflammation and neuronal apoptosis with enhanced proliferative activities in the hippocampus of rats exposed to chronic intermittent hypoxia. Thus, LBP may be proposed as a health supplement to mitigate neurological deficits in OSA patients, for which awaits future studies to delineate the neuroprotective mechanism of LBP. *[Studies supported by research grants (HKU 7510/06M, HKU 766110M) from RGC and funding (201007176007, SFPBR 200911159072) from HKU]*

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P44

THERAPEUTIC DEEP BRAIN STIMULATION IN PARKINSONIAN RATS DIRECTLY INFLUENCES MOTOR CORTEX

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Although deep brain stimulation (DBS) of the subthalamic nucleus (STN) is now a recognized therapeutic option for Parkinson's disease (PD), its exact mechanism of action is still not settled. Antidromic activation of the motor cortex from the STN has been hypothesized to contribute to the DBS effect but has not been demonstrated in freely moving hemi-Parkinson's rats and its mechanism remains obscure. Here, in the freely moving hemi-Parkinsonian rats, we identified short latency antidromic spikes in layer V corticofugal projection neurons (CxFn) during STN-DBS. Decreased success rate with increasing stimulation frequency produced the highest frequency of random antidromic spikes at 125Hz stimulation, which correlated with the optimal therapeutic efficacy. This effect was accompanied by increased firing rate, reduced pathological burst discharge and synchronization among the CxFn. Filed potential analysis revealed the renormalization of the pathological beta band oscillation and spike-field coherence during 125Hz STN-DBS. Importantly, we found evidence that the firing probability of the CxFn is modified following the occurrence of antidromic spikes, suggesting that direct interference of synchronized firing by stochastic antidromic spikes underlies the beneficial effect of STM-DBS. Our results therefore support that STN-DBS antidromically activates CxFn in the motor cortex through the cortico-subthalamic projection,

which directly disrupts abnormal neural activities in the motor cortex in PD.

P45

INCREASED SUSCEPTIBILITY OF α-SYNUCLEIN OVER-EXPRESSED *DROSOPHILA* TO IRON EXPOSURE IN THE PATHOGENESIS OF PARKINSONISM

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Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by selective loss of dopaminergic neurons and the presence of Lewy body inclusions in the substantia nigra of the midbrain. Although the etiology of PD remains incompletely understood, emerging evidence suggests that dysregulated iron homeostasis may be involved. α-synuclein is the major component of Lewy bodies, and point mutations of this gene are associated with familial PD. In an α-synuclein Drosophila model, loss of dopaminergic neurons, lamentous intraneuronal inclusions containing α-synuclein and motor dysfunction are found. However, how dysregulation of α-synuclein leads to neurodegeneration is not entirely clear. Here we hypothesize that interaction between iron and α-synuclein accelerates pathogenesis of Parkinsonism, and tested on a Drosophila model. Wild-type, α-synuclein normal (WT) and mutated (A53T/A30P) Drosophila were supplied with 15mM FAC (Fe3+) for up to 5 days. After the treatment, they were subjected to quantification of brain iron content, negative geotaxis assay for locomotor function and whole-mount immunostaining for dopaminergic neurons. While the brain iron content were increased in all groups, the death rate and motor dysfunctions were aggravated in the α-synuclein normal and mutated (A53T/A30P) Drosophila groups. Moreover, compared with the wild type control, α-synuclein normal (WT) and mutated (A53T) Drosophila, there was specific dopaminergic neuronal loss of PPM3 cluster in A30P mutated α-synuclein group after iron treatment. Taken together, these data reveal that α-synuclein expressed Drosophila exhibit greater susceptibility to iron accumulation, especially with respect to point mutation A30P.

Supported by the NSFC-RGC Joint Research Grant (N-CUHK433/08), National 973 Programs (2011CB510004), Direct Grant of CUHK (2011.1.084).

P46

THERPEUTIC EFFECT OF HEPCIDIN OVEREXPRESSION IN PARKINSION'S DISEASE MODEL

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Misregulation in brain iron homeostasis has been suggested to be a major cause in the pathogenesis of Parkinson's disease (PD). Thus, brain iron level is a potential target in the therapy of PD. It is well known that iron level in the periphery is regulated by hepcidin, the master iron regulatory hormone in general circulation. It is synthesized in the liver but is also expressed in the brain and therefore may contribute to brain iron homeostasis. In this study, we test the hypothesis that over-expression of hepcidin lowers brain iron content and is beneficial to parkinsonism, based on both *in vitro* and *in vivo* models.

In the *in vitro* studies, Mesencephalon (MES) 23.5 neurons were treated with different doses of 6-hydroxydopamine (6-OHDA) for 16 hours with or without pretreatment with hepcidin-adenovirus (Ad-hepcidin). The iron content and apoptotic level were increased by 6-OHDA treatment, which were reduced by over-expression of hepcidin via Ad-hepcidin. In the *in vivo* experiments, conventional PD model was generated in male Sprague Dawley rats by intranigral injection of 6-OHDA. One group of these animals was treated with Ad-hepcidin injection one week before 6-OHDA injection. While iron accumulation and neuronal degeneration were evident in the substantia nigra pars compacta of 6-OHDA injected group, in the Ad-hepcidin pre-treated group, these changes were dramatically reduced. Furthermore, the motor performance of the PD rats significantly improved, as assayed by the apomorphine-induced rotation.

Taken together, our data not only demonstrate that over-expression of hepcidin can regulate brain iron level but is neuroprotective and ameliorates parkinsonism motor symptoms. Manipulation of hepcidin

level thus has a potential to be developed as a novel therapy for PD.

Supported by the NSFC-RGC Joint Research Grant (N-CUHK433/08), National 973 Programs (2011CB510004), Direct Grant of CUHK (2011.1.084).

P47

ROLE OF ENDOCANNABINOID AND $GABA_A$ RECEPTOR IN MODULATING SYNAPTIC PLASTICITY IN THE VESTIBULAR NUCLEUS OF POSTNATAL RATS

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Endocannabinoid (eCB) has emerged as the key form of activity-dependent long-term presynspatic plasticity in the amygdala, hippocampus and visual cortex. eCB receptors are found to express in the vestibular nucleus but the role of eCB on long-term depression of inhibitory responses (iLTD) in central vestibular neurons remains unaddressed. Whole-cell patch-clamp experiments were therefore conducted on medial vestibular neurons in brainstem slices of postnatal rats (P5─15). iLTD of $GABA_A$ receptor-mediated evoked-IPSCs could be induced in these central vestibular neurons with theta-burst stimulation delivered to the vestibular afferents. In P5─7 rats, iLTD could be induced in 80% of the medial vestibular neurons tested. In P8─15 rats, however, iLTD could only be induced in 30% of the neurons tested. Application of antagonist of type 1 cannabinoid receptor (AM251) decreased the probability of iLTD induction to 30% in rats aged P5 to P7, while treatment with agonist of type 1 cannabinoid receptor (WIN55) increased the probability to 70% in rats aged P8 to P10. These results indicate that eCB modulates the efficacy of GABAergic synapses in the vestibular nucleus in an age-dependent role manner. [Supported by RGC 761710M]

P48

5-HT-TRIGGERED FACILITATION OF NEUROTRANSMITTER RELEASE IN MEDIAL VESTIBULAR NUCLEUS

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Serotonergic projections from the raphe nucleus are known to innervate the medial vestibular nucleus (MV). However, the role of this projection has not been determined. To address this issue, whole-cell patch-clamp and double-immunostaining methods were used to investigate the serotonergic modulation of spontaneous synaptic transmission within the MV of young rats. Perfusion of serotonin (5-hydroxytryptamine, 5-HT) to brainstem slices greatly facilitated spontaneous inhibitory postsynaptic currents (sIPSCs) in a subgroup of MV neurons. That this enhancement effect of 5-HT on sIPSCs was abolished with blocker of spontaneous action potentials indicates that 5-HT acts on the somatodendritic area of presynaptic neurons. Using $GABA_A$ or glycine receptor antagonist, we further identified that both receptors took part in the enhancement effect of 5-HT on sIPSCs. This facilitatory effect could be mimicked with the administration of $5\text{-}HT_2$ receptor agonist but was blocked with $5\text{-}HT_{2A}$ receptor antagonist. Similar operation was observed for serotonergic regulation of spontaneous postsynaptic currents (sEPSCs) in the MV circuitry. 5-HT significantly increased both the amplitude and frequency of sEPSCs. This 5-HT-induced enhancement of sEPSCs also depended on action potential. Such an enhancement of sEPSCs disappeared with AMPA receptor antagonist. $5\text{-}HT_2$ receptor agonist could mimic the 5-HT-induced facilitatory effect whereas use of a $5\text{-}HT_{2A}$ receptor antagonist blocked the effect. Our results therefore indicate that within the MV, $5\text{-}HT_{2A}$ receptors expressed on presynaptic neurons function to facilitate both inhibitory and excitatory neurotransmitters release.

P49

NEONATAL PERTURBATION OF VESTIBULAR GABAERGIC TRANSMISSION ALTERS SPATIAL NAVIGATION BEHAVIORS IN A RAT MODEL OF DEAD RECKONING

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Rodents navigate their surroundings using cues derived from the environment and from self-movement. To assess the role of vestibular information in providing idiothetic cues for spatial navigation, dead reckoning which requires the integration of cues on direction and distance for path-finding tasks was studied. We have reported previously that changes in GABAergic synaptic efficacy could be elicited in the vestubular nucleus during a limited time window of postnatal development. To study the role of GABAergic transmission in the developing vestibular nucleus on the acquisition of spatial recognition, we implanted above the vestibular nucleus of postnatal day 1 (P1) rats with a slice of Elvax loaded with $GABA_A$ receptor agonist (muscimol) or $GABA_A$ receptor antagonist (bicuculline). These animals were allowed to recover and were tested for dead reckoning at adult stage. When compared with the sham control group, rats treated with muscimol at P1 had a shortening of both the training days and searching time in light probe, dark probe and new location probe. On the other hand, treatment of bicuculline at P1 significantly prolonged the searching time in dark probe and new location probe. Besides, treatment of either muscimol or bicuculline had no evident effect on homeward time. Taken together, our data suggest that neonatal perturbation of GABAergic transmission in the vestibular nucleus impacted on the expression of vestibular-related spatial navigation behaviors. *[Supported by RGC 761711M]*

P50

INFLAMMATION-INDUCED UP-REGULATION OF BRAIN HEPCIDIN EXPRESSION

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Hepcidin, the recently discovered iron-regulatory hormone, is the central regulator of iron homeostasis. It regulates iron absorption in the intestine, iron recycling in the spleen, iron storage in the liver and iron utilization in the bone marrow via inducing internalization and degradation of ferroportin, the only known iron exporter. In the periphery, hepcidin expression is regulated by iron status, inflammation, erythropoiesis and hypoxia. Although it is predominantly produced by the liver, hepcidin is also distributed widely throughout the brain suggesting an essential role for hepcidin in brain iron metabolism. However, the mechanism by which hepcidin in the brain is regulated remains unknown. In this study, I investigated whether inflammation regulated hepcidin expression, which in turn could alter brain iron metabolism. A widely used inflammatory stimulus, lipopolysaccharides (LPS), was used to trigger neuroinflammation. In vivo, intracerebroventricular (i.c.v.) injection of LPS to rat brain induced brain hepcidin expression. In vitro, LPS was treated on primary neurons. Intriguingly, LPS did not affect hepcidin expression in primary neurons, however, LPS induced hepcidin in primary neurons with the presence of BV-2 microglia. Further investigation of effects of a series of pro-inflammatory cytokines which could be released by BV-2 microglia, on hepcidin expression in neurons, demonstrated that among these cytokines, only IL-6 increased neuronal hepcidin expression. As a validation of this, anti-IL-6 antibody blocked LPS-induced neuronal hepcidin expression. These results suggested that IL-6 mediated LPS-induced hepcidin expression in neurons with the presence of microglia. Furthermore, I found that IL-6 induced hepcidin up-regulation (including expression and release) and ferroportin down-regulation most significantly at 2 hours. By using a STAT3 inhibitor, stattic, it was found that STAT3 was the downstream signalling of IL-6-induced hepcidin up-regulation and ferroportin down-regulation in primary neurons. My findings suggest that brain hepcidin is up-regulated by inflammation.

Supported by the NSFC-RGC Joint Research Grant (N-CUHK433/08), National 973 Programs (2011CB510004), Direct Grant of CUHK (2011.1.084).

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EFFECTS OF HYPOOSMOLARITY ON CANONICAL TRANSIENT RECEPTOR POTENTIAL 5 ACTIVITY IN RAT AORTIC ARCH BARORECEPTOR NEURONS.

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Aortic arch baroreceptor is a major arterial baroreceptor which plays an essential role in regulation of arterial blood pressure. Yet the molecular identity of the aortic arch baroreceptor sensor remains unclear. Canonical Transient Receptor Potential 5 (TRPC5) is a member of the seven mammalian TRPC cationic channels respond to mechanical stress including hypoosmolarity. It was found that TPRC5 is expressed in aortic arch baroreceptor neurons. We examined the hypothesis that TPRC5 was involved in pressure sensing in baroreceptor neurons. Whole-cell patch clamping study was performed on the cultured rat nodose baroreceptor neurons isolated from the left nodose ganglion. Gadolinium ion (Gd^{3+}, 20mM) was included in the bath solution to potentiate TRPC5 activity and to block other cation channels. A whole-cell cationic current was substantially increased in hypotonic condition (240mOsm) when compared with isotonic condition (300mOsm). Pretreatment of anti-TRPC5 blocking antibody T5E3 (15mg/ml) abolished the hypoosmolarity-activated current, whereas perimmmune IgG (15mg/ml) had no effect. To further verify the mechanosensitivity of TRPC5, the nodose ganglion neurons were transiently tranfected with TPRC5 (6mg/ml) by electroporation. Hypoosmolarity stimulated a whole-cell cation current in TRPC5-overexpressing neurons. Pretreatment of TRPC5 inhibitor 2-aminoethoxydiphenyl borate (2APB, 100mM, 10 min) markedly reduced this whole cell current. Taken together, these results suggest that TRPC5 is involved in mechanosensing at the aortic arch baroreceptor.

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AGE-DEPENDENT CHANGES OF IRON-RELATED PROTEINS IN RAT BRAIN

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One of the common age-dependent changes identified in human is brain iron elevation in regions that are also affected in many neurodegenerative diseases, such as Alzheimer's and Parkinson's disease. In this study, in an attempt to achieve a more systematic and holistic investigation, we studied the effect of age on iron, cellular distribution of various iron-related proteins and cell morphology in different brain regions obtained from 3 month-old (mth), 11 mth and 24 mth rats. Total iron measurement by furnace atomic absorption spectrometry revealed elevated iron level, from 3 mth to 24 mth, in the striatum, hippocampus and cortex. Enhanced Perls' iron staining showed that both cytoplasmic iron and extracellular iron increased with age in the substantia nigra, striatum, hippocampus and cortex, in decreasing order. Semi-quantitative analysis of the immunohistochemistry results suggests that DMT1(+IRE) level first decreased and eventually increased in hippocampus but kept increasing with age in substantia nigra, striatum and cortex. TfR level first remained unchanged and eventually increased in substantia nigra but kept decreasing with age in hippocampus and increasing with age in striatum and cortex. DMT1(-IRE), CP and Fpn level were all found to increase with age in all of the 4 regions examined. Cell morphology in striatum and cortex were found to be mostly conserved while cell shrinkage, which was previously found to be associated with surviving neurons that were affected by iron accumulation, was frequently observed in substantia nigra and occasionally in hippocampus at 24 mth. These results suggest that age has a significant effect on brain iron level, iron-related protein level, and cellular morphological alteration especially in substantia nigra. Further investigation is needed to confirm the suspected protein level alteration and evaluate the relative contribution of iron-related proteins to age-dependent iron elevation and cellular deterioration in normal brain.

Supported by the NSFC-RGC Joint Research Grant (N-CUHK433/08), National 973 Programs (2011CB510004), Direct Grant of CUHK (2011.1.084).

P53

AMPAKINES RESCUE CHRONIC INTERMITTENT HYPOXIA-INDUCED IMPAIRMENT IN HIPPOCAMPAL SYNAPTIC PLASTICITY AND MEMORY DEFICIT IN THE MOUSE

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Obstructive sleep apnea (OSA) is a common sleep and breathing disorder resulting in intermittent hypoxia (IH), and can cause neurocognitive deficits including impairment in attention, planning and memory. It is well known that learning and memory involves long-term potentiation (LTP), a form of synaptic plasticity. Our recent work has shown that intermittent hypoxia (IH) impairs both early phase LTP (E-LTP) and late phase LTP in the hippocampus, and accompanied by a reduction in the level of brain-derived neurotrophic factor (BDNF). In this study, we examined the effects of administration of ampakine, a group of AMPA receptor modulator known to elevate endogenous BDNF level by short-term administration. Two groups of adult male mice were exposed to 7-day IH (90s cycles between 10% and 21% O_2 levels for 8 hrs) and received vehicle and ampakine injection respectively from day 4 to day 7 while another group under normoxia served as control. We found that there was a significant increase in E-LTP in ampakine injection group (16 slices, 5 mice, 156.5±7.4%) compared with the vehicle-treated group (13 slices, 4 mice, 133.3 ± 7.9%; $P < 0.05$), and was similar to that of the normoxia group (6 slices, 4 mice, 159.1±7.6%; $P > 0.05$). Ampakine treatment also restored the decreased level of hippocampal BDNF in the IH-treated group, as revealed by Western blot. Furthermore, in radial arm maze test, ampakine administration improved the long-term reference memory of the IH mice. Together, these data confirm the role of BDNF in chronic IH and that ampakine has therapeutic value for the neurocognitive symptoms of OSA subjects. *(Supported by GRF Grant 478308 & Joint NSFC/RGC Grant N_CUHK453/09)*

P54

WHOLE-CELL CURRENTS ACTIVATED BY GLYCINE IN GLOBUS PALLDIUS OF THE YOUNG RAT

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Globus pallidus (GP), as one of major output nuclei of the basal ganglia circuitry, plays a significant role in regulating motor functions, and has been implicated in motor disorders such as Parkinson's disease. GABA-A receptors are considered to be the major receptor in controlling the neuronal activities of GP neurons. However, neuroanatomical findings revealed the existence of glycine receptors in this region, although their functions have not been elucidated. Here, using whole-cell patch-clamp recordings, we investigated the electrophysiological effects of glycine and also the properties of glycine receptor-mediated synaptic currents in GP of young rats.

Two types of GP neurons were distinguished mainly based on the presence (type A) or absence (type B) of hyperpolarization-activated inward current. Both types of neurons stained positively for glycine receptors, and responded to the application of both glycine and GABA by generating an outward current that inhibited the firing of these neurons. The specificity of glycine on glycine receptor was confirmed by the antagonistic action of strychnine but not picrotoxin. Interestingly, the current evoked by glycine in type A neuron was significantly larger than that evoked in type B neuron while these two types of neurons responded similarly to GABA. Finally, while most of the spontaneous IPSCs (sIPSCs) appeared to be mediated by GABA-A receptors, a small portion of the sIPSCs were mediated by strychnine-sensitive glycine receptors. In conclusion, despite that GABA-A receptor is the major receptor mediating the inhibitory action originating from the striatum or from local neurons, glycine and glycine receptor may play a specific role in modulating the neuronal activities in different subtypes of GP neurons. These findings may have implications in understanding the etiology of GP-related motor disorders.

P55

ALTERATION OF CORTICOSTRIATAL GLUTAMATERGIC TRANSMISSION ONTO D2 MSNS IN ANIMAL MODELS OF PARKINSON'S DISEASE

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Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by motor

symptoms and nonmotor symptoms, which are mostly resulted from progressive death of dopaminergic neurons in the midbrain. The pathophysiological mechanisms are not thoroughly understood. A recent study reports that spines are selectively eliminated in striatal D2 receptor-expressing medium spiny neurons (MSNs) but not in D1 MSNs in the parkinsonian states. We wonder whether the corticostriatal transmission is affected in relation to different neuronal types. By taking advantage of bacterial artificial chromosome D2 transgenic mice, in which enhanced green fluorescent protein is expressed under the control of D2 receptor promoter, we compared paired-pulse ratios (PPRs) of cortically evoked excitatory postsynaptic currents in D1 MSNs and D2 MSNs in slice preparations from naïve and mouse models of PD using whole-cell patch-clamp recordings. Our results showed that corticostriatal AMPA PPRs and NMDA PPRs were specifically decreased in D2 MSNs, but not in D1 MSNs, following dopamine depletion. In the presence of cyclothiazide to block AMPA receptor desensitization, bath application of γ-DGG detected higher glutamate content in the corticostriatal synaptic cleft associated with D2 MSNs from dopamine-depleted mice, which was not caused by malfunction of glutamate transporters. All these observations suggested an increase in the corticostriatal glutamate release onto D2 MSNs in the parkinsonian states. This enhanced glutamate release further led to an increase in the AMPA receptor occupancy level and subsequently in the AMPA receptor desensitization in D2 MSNs. In conclusion, the corticostriatal glutamate release onto indirect-pathway striatal projection neurons was selectively increased in the parkinsonian states, which may underlie the motor deficits in PD.

P56

DERIVATION OF OLIGODENDROCYTES PRECURSORS FROM BONE MARROW STROMAL CELLS FOR MYLINATION THERAPY

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Myelin damage and disorders caused by physical damage or diseases like leukodystrophies result in severe loss of function. With an aim towards remyelination therapy, we attempted to direct differentiation of bone marrow stromal cells (BMSCs, adult rats) along the oligodendroglial lineage *in vitro*. BMSCs were first cultured as non-adherent spheres until they expressed markers of neural/glial progenitors. The neural/glial progenitors were then maintained in adherent culture supplemented with β-heregulin, PDGF-AA and bFGF. Oligodendrocyte precursors expressing the markers - NG2, Olig2, PDGFRa and Sox10, were detectable within two weeks and can be expanded in culture for up to 3 months with no observable decline in marker expression. To test for myelination capability, BMSC-derived oligodendrocyte precursor cells (OPCs) in a 2-week co-culture with dorsal root ganglion neurons extended myelin basic protein-positive processes along neurites, suggesting maturation into myelinating oligodendrocytes. *In vivo* myelination by BM-OPCs was tested by exploitation of unmyelinated axons of retinal ganglion cells of adult rats. Myelin basic protein-positive processes were also observable along retinal axons by 8 weeks post-injection. Our findings indicate BMSCs as a possible source of OPCs for remyelination therapy.

P57

THE MECHANISMS OF CHONDROITIN SULPHATE LYASES TREATMENT IN PROMOTION OF AXONAL GROWTH.

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In Injured nerves, chondroitin sulphate (CS) is upregulated forming barriers with astrocytes/fibroblasts and other extracellular matrix molecules, and thereby hampering nerve regeneration. Cleavage of CS using chondroitin sulphate lyases (*Proteus vulgaris*) promises axon regrowth through the barrier but the enzymatic efficacy remains to be improved. Two subtypes, endolyase and exolyase, have been found coexisting in the original host but only the former has been exploited for treatment of injured nerve tracts.

We hypothesise that the two subtypes are necessary for enzymatic efficacy on CSs. We therefore prepared recombinant enzymes of both subtypes. Enzyme kinetics study revealed feedback inhibition by limit digestion products: that of the endolyase by tetrasaccharides and that of the exolyase by the disaccharides. When the two subtypes were used in combination, the digestion efficiency increased. We then used TGF beta-1 to induce CS production by astrocytes in culture, mimicking reactive glia in injured nerves. In co-cultures of such astrocytes with cortical neurons, treatment with combinations of the two subtypes resulted in increased neurite lengths as compared to co-cultures treated with one of the subtypes. The limit digestion products of CS were further tested for their effects on neurite extension on astrocytes that had been treated with TGF beta-1. The CS disaccharides, both 4- and 6-sulphated but not the tetrasaccharides, promoted neurite extension significantly. Taken together, the combinatorial use of the ChABC subtypes not only improved efficacy of enzyme activity on the axon-restrictive CS moiety, but also increased the yield of CS disaccharides which contributed to axonal growth.

P58

ROLES OF PERINEURONAL MATRIX IN DEVELOPMENTAL PLASTICITY OF THE CENTRAL VESTIBULAR SYSTEM

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Perineuronal nets (PN), as detectable by *Wisteria floribunda* agglutinin (WFA) staining, are extracellular matrix molecules surrounding GABAergic neurons. We hypothesize that PN regulate developmental plasticity of central vestibular circuitry containing GABAergic neurons. To address this, we looked for changes in PN during postnatal development of the central vestibular system. WFA-stained PN in the vestibular nucleus (VN) changed from diffuse organization in neonates to consolidated network in adults. Previous studies suggested that PN formation is activity-dependent. Therefore, the effect of vestibular deprivation on PN development in the VN was also investigated. Following bilateral labyrinthectomy (BL) at P3, the number of VN neurons with PN at adult stage was less than in normal controls. However, following BL at P7 or later, the number of PN-bound VN neurons were similar to normal controls. Our findings indicate that the first postnatal week is a critical period during which inputs from the peripheral afferents provide the necessary trigger for PN formation. The relationship between PN formation and maturation of vestibular functions was further studied with a behavioral test for negative geotaxis (a vestibular reflex). Normal rats acquired the mature response at P9, the time when PN consolidation was first observed. However, the acquisition was delayed to P13 in rats pretreated with chondroitinase ABC injection into the VN at P6 to prevent PN formation. Therefore, PN consolidation in the VN is critical to timely acquisition of the vestibular reflex. Taken together, our results show that PN undergo activity-dependent reorganization that corresponds to the maturation of vestibular behavior, indicating that PN play important roles in plasticity of the developing central vestibular system. [*Supported by HKRGC*]

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REGULATORY ROLE OF PROHEPARANASE WITH PERI-SYNAPTIC HEPARAN SULFATE PROTEOGLYCAN AND AMPA-TYPE GLUTAMATE RECEPTOR IN SYNAPTIC PLASTICITY

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AMPA-type glutamate receptors (AMPAR) govern excitatory synaptic transmission. Perineuronal heparan sulfates (HS) have been implicated in controlling the open-state of AMPAR. Our finding of neuronal heparanase expression in adult rats led us to test (1) if neuronal heparanase is secreted and (2) if the secreted form acts on perineuronal HS to modulate synaptic plasticity.

Neuronal secretion of heparanase was triggered by phorbol ester of rat hippocampal neurons in culture. Western blot analysis of the secreted product revealed enzymatically inactive proheparanase, but not the enzymatically active heparanase. Synaptosomes prepared from phorbol ester-treated rat cortex

slices showed enrichment in proheparanase; co-immunoprecipitation studies further showed association of AMPAR subunits (GluA1 and GluA2/3) with both syndecan-3 (a transmembrane HS-proteoglycan) and proheparanase, suggesting their partnership in the peri-synaptic environment. Treatment of hippocampal neurons in culture with recombinant proheparanase triggered internalization of proheparanase, perineuronal HS-proteoglycans and AMPARs, suggesting their clustering as a functional complex. Heparitinase pre-treatment of hippocampal neuron cultures reduced proheparanase-induced internalization of AMPARs, suggesting that the HS moiety is critical for effecting the partnership. Treatment of hippocampal slices with recombinant proheparanase resulted in down-regulation of both basal synaptic strength and LTP at Schaffer collateral synapses. These results reveal a novel role of neuronal proheparanase in resetting AMPAR and perineuronal HS levels at the synapse and thus the modulation of synaptic plasticity.

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NOTCH SIGNALING MEDIATES THE SWITCH TO FATE COMMITMENT OF BONE MARROW-DERUVED SCHWANN CELLS

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Our strategy of deriving fate-committed Schwann cells from bone marrow stromal cells (Shea et.al, 2010) supports that the cell-intrinsic switch to fate commitment depends on developing neurons of sensory ganglia. We therefore hypothesize that neurons recoverable from dorsal root ganglia (DRG, E14/15 rats) are at a stage that provides the juxtacrine/paracrine signals for the switch. Analysis of the DRG neurons found cell surface immunopositivities for DLL1, Jagged1 (ligands of Notch receptor) and Neuregulin-1 type III (ligand of ErbB receptors). While bone marrow-derived Schwann cell-like cells (SCLC) were immunopositive for Notch-1, coculture with DRG neurons resulted in progressive increase in ErbB2/B3 expression as revealed by immunocytochemistry and Western blotting. Following activation of the Notch1 receptor – Western blot revealed elevated level of Notch intracellular domain (NICD) during the switch to fate commitment; the level returned to basal when the bone marrow-derived cells tested true for commitment to the Schwann cell fate and in vitro myelination. Our results indicate that DRG neuron-SCLC interaction accomplishes DLL1/Jagged1 -Notch signaling that mediates SCLC expression of the ErbB receptors for signaling interaction with neuregulin-1 type III of DRG neurons. The findings promise the prospect of abundant fate-committed Schwann cells generated from an autologous source for use in nerve repair and regeneration.

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SIGNAL INVOLVED IN THE SWITCH TO FATE COMMITMENT OF BONE MARROW-DERIVED SCHWANN CELLS

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Schwann cell transplantation can promote axonal regrowth and remyelination following nerve injury. Our group has reported the generation of fate-committed bone marrow-derived Schwann cells by co-culturing with dorsal root ganglia (DRG, E14/15 rats) neurons (Shea *et. al*, 2010). Juxtacrine/paracrine signals from DRG neurons are therefore thought to provide instructive cues to direct the switch of bone marrow-derived Schwann cell-like cells (SCLC) to fate committed Schwann cells. We hypothesized that neuregulin 1 Type III (Nrg 1 Type III), the membrane bound form of Nrg contributes to the switch but not the soluble isoforms. SCLC treated with soluble Nrg did not show significant changes in morphology nor marker expression when compared with untreated SCLCs. Purified DRG neurons were found to express Nrg 1 Type III as indicated by immunocytochemistry and polymerase chain reaction for the mRNA. A Nrg 1 Type III construct was also made for transfection into HEK 293T and mouse embryonic fibroblasts (MEF)

such that these could be tested as surrogate cell types in co-culture with SCLCs to pursue cell-specific effects of Nrg 1 Type III on differentiation of SCLCs. The findings promise a way for generating fate committed Schwann cells for autologous transplantation into nerve lesion sites to promote functional recovery.

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IMMOBILIZATION OF CHONDROITINASE ABCI ON CHITOSAN BEADS TO IMPROVE AXONAL REGROWTH IN CSPG-ENRICHED ASTROCYTE CULTURE

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After nerve injury, scar tissue enriched in chondroitin sulfate proteoglycans (CSPGs) is often formed to contain the lesion but is restrictive to axonal regrowth. Chondroitinase ABCI (ChABCI) has been exploited to cleave CS moieties of the PGs and thus to enhance prospects of axonal regrowth through the lesion. ChABCI activity decay in vivo has however hindered application of the enzyme in nerve regeneration. We attempted to address this by immobilization of recombinant ChABCI on a selected matrix. Chitosan was chosen because it is non-toxic, biocompatible and tunable in rate of biodegradation. We prepared chitosan beads (73.89μm±28.59) that precluded phagocytosis by inflammatory cells. ChABCI that was cross-linked to the chitosan beads under specified conditions demonstrated CS-cleaving activity both in biochemical assay and in astrocyte cultures that had been activated to secrete CSPGs. Factors that contribute to activity decay as thermal instability and susceptibility to end product inhibition are assessed. Axonal regrowth on activated astrocyte cultures was improved by co-treatment with ChABC I and ChABC II.

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PREPARATION OF CHITOSAN FIBERS FOR NERVE REGENERATION

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Schwann cells have been exploited to guide axonal regrowth after nerve injury. However, mis-orientation of Schwann cells in nerve guidance channels can hinder axonal growth across the lesion site. We therefore propose to orient the growth of Schwann cells on uniaxially aligned fibres such that axonal growth can be guided along the designated direction towards the target. Chitosan was the choice scaffold material given its biocompatibility and the tunable susceptibility to biodegradation. With a conventional electrospinning setup and chitosan dissolved in trifluoroacetic acid/methylene chloride, our initial attempts resulted in a random orientation of chitosan fibers at the cathodic collector. By replacing the grounded plate collector with parallel collector plates placed 1.6 cm apart, the positively charged chitosan fibers became alternately attracted to the parallel plates and ended up uniaxially aligned as fibre suspension across the plates. Stability of the chitosan fibers in aqueous, physiological environment was achieved with use of sodium carbonate to neutralize residual acidity in the chitosan fiber preparation. Cell culture experiments are ongoing to test if Schwann cell behavior varies with fiber thickness of the stabilized chitosan fiber preparations.

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ALDOSE REDUCTASE DEFICIENCY PROTECTS THE RETINAL NEURONS IN A MOUSE MODEL OF RETINOPATHY OF PREMATURITY

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Purpose: Retinopathy of prematurity (ROP) is a common retinal disease occurred in premature babies. It is found to be related to oxidative stress while dysfunction of the neural retina has also been documented. We previously showed that genetic deletion or pharmacological inhibition of aldose reductase (AR), a rate-

limiting enzyme in the polyol pathway, prevented ischemia-induced retinal ganglion cell (RGC) loss and oxidative stress. Here, we assessed the effects of AR deletion on retinal neurons using a mouse model of ROP. Methods: Seven-day-old mouse pups were exposed to 75% oxygen for five days and returned to room air. The pathological neuronal changes were examined and compared between wild-type (WT) and AR-deficient retinae on P14 and P17 (P, postnatal). Retinal thickness was measured and immunohistochemistry for calbindin, calretinin, PKCα, Tuj1, glial fibrillary acidic protein (GFAP), nitrotyrosine (NT), as well as poly(ADP-ribose) (PAR) was performed. Results: After hyperoxia exposure, significantly reduced inner nuclear layer (INL) and inner plexiform layer (IPL) thickness were found in both genotypes. The intensity of calbindin staining for horizontal cells in INL was reduced in the WT retinae but not in AR-deficient retinae. In addition, significant reduction was found in calretinin-positive amacrine cell bodies in central INL especially in WT retinae. Serious distortion was also observed in the three calretinin-positive strata along IPL in the WT retinae but not AR-deficient retinae on P17. Moreover, increased GFAP intensity across IPL indicating Müller cell processes was observed in AR-deficient retinae on P14 and in WT retinae on P17. Furthermore, increased NT immunoreactivity in INL and nuclear or para-nuclear PAR staining along GCL were observed in WT retina while these changes were not apparent in AR-deficient retina. Conclusion: Our observations demonstrated morphological changes of retinal neurons in the mouse model of ROP and indicated that AR deficiency showed neuronal protection in the retina, possibly through modulating glial responses and reducing oxidative stress.

P65

REDUCED EXPRESSION OF ENDOGENOUS BRAIN-DERIVED AND GLIAL CELL-LINE DERIVED NEUROTROPHIC FACTORS IN THE REACTIVE ASTROCYTES OF THE OVARIECTOMIZED PARKINSONIAN RAT

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We have previously reported an unknown self repair mechanism during extremely early stages of rat Parkinsonism (Lui et al; Program No. 460.20. 2010 Neuroscience Meeting Planner. San Diego, CA: Society for Neuroscience, 2010. Online). In the striatum of 6-hydroxydopamine-lesioned female rat, nestin-positive reactive astrocytes appeared at post-lesion day 3 while very low levels of brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) dimers, which were believed to initiate the signal transduction cascades for anti-apoptotic actions, were found. Peak levels of BDNF and GDNF found at post-lesion day 7 while nestin-positive astrocytes had started to disappear. In the substantia nigra, the specific patterns of nestin and BDNF expressions were similar to those of the striatum except no GDNF could be detected during the whole period. At post-lesion day 14, expressions of nestin-positive reactive astrocytes, BDNF and GDNF were curtained. Here we further investigate the neurotrophic roles of the reactive astrocytes in the 6-hydroxydopamine-lesioned ovariectomized rat. In the study, we found that the expressions of neurotrophic factors were reduced in the nestin-positive reactive astrocytes of both striatum and substantia nigra. The up-regulation of BDNF appeared at post-lesion day 5 and day 7 only. No expression of GDNF could be revealed throughout the studied period. Changes in the levels of estrogens may regulate the expression of neurotrophic factors and thus affect the neuroprotective activities of the reactive astrocytes on the dopaminergic neurons.

P66

BRAIN-DERIVED NEUROTROPHIC FACTOR IS EXPRESSED IN REACTIVE ASTROCYTES IN VITRO AND PROVIDES NEUROPROTECTION TO SH-SY5Y CELLS AGAINST 6-HYDROXYDOPAMINE TOXICITY

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Brain-derived neurotrophic factor (BDNF) has been shown to enhance the survival of dopaminergic neurons and to protect them against the neurotoxic effects of 6-hydroxydopamine (6-OHDA), a specific neurotoxin to dopaminergic neurons (Bove et al., 2005). Nestin is an embryonic protein and is a marker for

neural stem cells. Nestin is not found in mature astrocytes. In our previous studies using rodent models of Parkinson's disease, a few nestin-immunoreactive reactive astrocytes were found to appear and express BDNF after the onset of Parkinson's disease (Chung et al., 2008). In order to investigate the neuroprotective roles of BDNF in reactive astrocytes, primary cell cultures of rat astrocytes were employed in the present study. By immunocytochemistry and Western blot analysis, a vast proportion of astrocytes in culture were found to be nestin-immunoreactive. In addition, most of these reactive astrocytes were found to express BDNF immunoreactivity. Reactive astrocytes were treated with BDNF (5, 50, 100, 200 ηg/ml). The levels of BDNF expression were found to be further enhanced and up-regulation of p-MEK and p-Erk1/2 in astrocytes were found by Western blot analysis. Moreover, when BDNF treated reactive astrocytes were co-cultured with neuroblastoma SH-SY5Y cells, activation of Akt and MAPK pathway were found by Western blot analysis. Cells in co-cultures were then treated with 6-OHDA (40μM), significant reductions of cell death of SH-SY5Y cells were found. The present results as a whole indicate that astrocytes in vitro display characterizations of reactive astrocytes and express high levels of BDNF. Administration of BDNF can stimulate further expression of BDNF in reactive astrocytes through activation of the MAPK pathway.

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ENHANCEMENT OF GENE SILENCING EFFECTS OF SMALL INTERFERING RNAS TO NMDA RECEPTORS BY NANOPARTICLES

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Parkinson's disease (PD) results from the loss of nigrostriatal dopaminergic neurons in substania nigra pars compacta (SNpc). Over-stimulation of glutamate receptor by glutamate results in excitotoxic cell death that is a major form of dopaminergic cell death in PD. N-methyl-D-aspartate (NMDA) receptor is an ionotropic glutamate receptor consists of subunits including NR2B and they are the key receptors that mediate excitotoxic cell death. In the present study, NR2B specific small interfering RNA (siRNA) was designed to undergo gene silencing in SH-SY5Y neuroblastoma cells. The reduction of protein expression of NR2B may therefore lead to a decrease in expression of functional NMDA receptor. Excitotoxicity and cell damage should be reduced. However, efficiency of gene transfection by siRNA is limited and the efficacy is not high. In order to enhance transfection efficiency, gold nanoparticles coated with polyethylene glycol with siRNA embedded (Au-PEG-siRNA) was made for gene silencing. Thiol group attached with polyethylene glycol (PEG-SH) was added to gold nanoparticles. Au-PEG was held together by strong disulfide linkage. The PEG polymer shielded siRNA from degradation. Results showed Au-PEG-siRNA enhanced NR2B specific siRNA transfection by decreasing NR2B expression significantly in both immunocytochemistry and Western blot analyses. Au-PEG also showed low cytotoxicity to SH-SY5Y cells. In addition, neuroprotective effects of the Au-PEG-siRNA were also investigated by toxicity assays using glutamate and NMDA. No significant change in percentage of cell death was found with Au-PEG-siRNA pre-treatment followed by NMDA treatment. No significant change was observed in the levels of p-akt(Ser473) protein in the NMDA treated cells. However, cytotoxicity was found to reduce in SH-SY5Y cells with Au-PEG-siRNA pre-treatment followed by 10mM L-glutamate treatment. The levels of p-akt (Ser473) proteins were significantly up-regulated in Au-PEG-siRNA pretreated group. Results of the present study indicate that Au-PEG enhances the efficiency of gene silencing of siRNA. Neuroprotection resulted by Au-PEG-siRNA application may be mediated through the Akt cell survival signaling pathway.

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THE GAIN MODULATION BY N-METHYL-D-ASPARATE IN THE PROJECTION NEURONS OF ROBUST NUCLEUS OF THE ARCOPALLIUM IN ADULT ZEBRA FINCHES

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The song of zebra finch is stable in life after it was learned successfully. Vocal plasticity is thought to be a motor exploration that can support continuous learning and optimization of performance. The activity of

RA, an important pre-motor nucleus in songbird's brain, influences the song directly. This variability in adult birdsong is associated with the activity of NMDA receptors in LMANRA synapses, but the detailed mechanism is unclear. The control of gain refers to modulation of a neuron's responsiveness to input and is critically important for normal sensory, cognitive, and motor functions. Here, we observed the change of gain in RA projection neurons after exogenous NMDA was applied to activate NMDA receptors using the whole-cell current clamp recording. We found that NMDA substantially increased the slope (gain) of the firing rate-current relationship in RA projection neurons. The AMPA receptor-dependent excitability played a crucial role in the modulation of gain by NMDA. These results suggested that NMDA receptors may regulate the dynamics of RA projection neurons by input-output gain.

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MELATONIN INDUCES FHIT EXPRESSION AND PHOSPHORYLATION THROUGH G16

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Melatonin, a neuroendocrine hormone produced in the pineal gland, is important for the regulation of seasonal and circadian rhythms, body temperature, and immune function in mammals. Melatonin exhibits these biological activities through its anti-oxidative function, as well as acting on its nuclear receptor and G protein-coupled transmembrane receptors. There are two types of G protein-coupled melatonin receptors (MT_1 and MT_2) in human, and both of them are coupled to the G_i proteins for adenylyl cyclase inhibition. Our previous studies have demonstrated that they are also capable of activating phospholipase Cb through G_{16}, a G_q family member specifically expressed in immuno-responsive cells. It has been reported that activation of melatonin MT_1 receptor suppressed the proliferation rates of certain cancer cell types. Coincidently, our recent study revealed that G_{16} may regulate Fhit (fragile histidine triad protein), an Ap3A hydrolase with tumor suppressor activity. Hence, melatonin may modulate the cell proliferation via its regulation on Fhit through G_{16}. Here we demonstrated that the functional coupling between G protein-coupled melatonin MT_1 receptor and G_{16} resulted in an increased expression and Tyr^{114} phosphorylation of Fhit, while the MT_1 receptor-G_i coupling was ineffective in eliciting the same responses. We further proved that the up-regulated Fhit expression depends on the increased Fhit protein synthesis in the ribosome via the PKC/MEK pathway, and Src participates in the melatonin-induced Fhit phosphorylation. Such findings illustrate the importance for investigating the roles of Fhit, in terms of its possible influences on the neuro-endocrinology of immune cells expressing both melatonin MT_1 receptor and G_{16}. *Supported by the Research Grants Council of Hong Kong (660108 and 663108).*

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EFFECTS OF TROPHIC STIMULATION ON THE MATURATION OF THE NEUROMUSCULAR JUNCTION

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The neuromuscular junctions (NMJs) undergo maturation process after the initiation of nerve-muscle interaction. Physiologically, An increase in the spontaneous synaptic current (SSC) and a decrease in the SSC rise time were detected during the first three days. Meantime, there was a shift of the SSC amplitude histograms from skewed to bell-shaped distribution. Morphologically, consolidated nerve-induced postsynaptic AChR clusters as well as enhanced apposition of pre- and postsynaptic specializations along nerve-muscle contact were observed. Interestingly, chronic application of brain-derived neurotrophic factors (BDNF) or elevation of intracellular cyclic AMP (cAMP) was able to inhibit the maturation processes. Significant suppression was observed in the physiological and morphological properties comparing to the control groups. Our previous studies have suggested that BDNF and cAMP enhance the survival and growth of spinal neurons but inhibit synaptogenesis. These results further demonstrate the negative role of BDNF and cAMP on NMJ maturation. *(Supported by Hong Kong RGC GRF grant 662311)*

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TIMING AND ROUTES OF ENTRY OF MICROGLIAL PROGENITORS TO THE EMBRYONIC CENTRAL NERVOUS SYSTEM

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Microglia are resident phagocytes within the central nervous system (CNS), and their progenitors enter the CNS during the embryonic period. However, the timing and routes of their entry to the CNS are not fully understood. In this study, we used Iba1 (ionized calcium binding adaptor molecule 1) as a specific marker to label microglial progenitors in mouse embryos and employed multiple immunofluorescence localization and transplantation to establish (a) the timing and (b) the routes of their entry to the neural tube (developing CNS). At E9.5, $Iba1^+$ microglial progenitors were found in the mesenchyme, but no immunoreactive cells were found within the neuroepithelium of the neural tube. Half a day later at E10.0, a few $Iba1^+$ cells started to appear within the neuroepithelium. By E10.5, more $Iba1^+$ cells were found within the neuroepithelium and the embryonic liver. Upon careful examination of the distribution of $Iba1^+$ cells, we found that some $Iba1^+$ cells straddled the apical or basal surface of the neuroepithelium, and some were located at the wall of blood vessels. The number of $Iba1^+$ cells within the neuroepithelium was increased dramatically from E10.5 to E13.5. When dissociated E11.5 liver cells genetically labelled with eGFP were transplanted to the mesenchyme at E10.5, $eGFP^+Iba1^+$ cells were found within the neuroepithelium one day later, implicating that transplanted liver cells were able to migrate through the mesenchyme to the neural tube to form $Iba1^+$ microglial progenitors. Our results suggest that microglial progenitors start to enter the developing CNS at around E10.0 through the apical or basal side of the neural tube or through blood vessels, and the embryonic liver could represent a source of microglial progenitors from E10.5.

This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project no. CUHK461909).

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STRUCTURE-FUNCTION STUDY OF UBIQUITIN C-TERMINAL HYDROLASE L1 (UCH-L1) BY NMR SPECTROSCOPY – INSIGHTS INTO UCH-L1 MUTATION'S ASSOCIATION WITH THE RISK OF PARKINSON'S DISEASE

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Protein ubiquitination and deubiquitination, play important roles in many aspects of cellular mechanisms. Its defective regulation results in diseases that range from developmental abnormalities to neurodegenerative diseases and cancer. Ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) is a protein of 223 amino acids, which is highly abundant in brain, constituting up to 2% of total brain proteins. Although it was originally characterized as a deubiquitinating enzyme, recent studies indicate that it also functions as a ubiquitin ligase and a mono-Ub stabilizer. Down-regulation and extensive oxidative modifications of UCH-L1 have been observed in the brains of Alzheimer's disease and Parkinson's disease (PD) patients. Of importance, I93M and S18Y point mutations in the UCH-L1 gene have been reported to be linked to susceptibility to and protection from PD respectively. Hence, the structure of UCH-L1 and the effects of disease associated mutations on the structure and function are of considerable interest.

Our circular dichroism studies suggest that the S18Y point mutation only slightly perturbs the structure while a significant decrease in the α-helical content is observed in the I93M mutant. We have determined the solution structure of S18Y and mapping its interaction with ubiquitin by chemical shift perturbation approach. The electrostatic surface potential analysis reveals that the interaction between ubiquitin and UCH-L1-S18Y is primarily electrostatic in nature, with negatively charged residues on the surface of UCH-L1-S18Y interacting with the positively charged residues on the basic face of ubiquitin.

Although the active site and the L8 loop in UCH-L1-S18Y adopts conformations similar to that observed in the crystal structure of UCH-L1-WT, both the altered hydrogen bond network and surface charge distributions have demonstrated that the S18Y substitution could lead to profound structural changes. In particular, the difference in the dimeric interfaces of the wild-type and the S18Y mutant has shown that mutation can significantly affect the distribution of the surface-exposed residues involved in the dimeric interface. Such observed difference might weaken the stability of the UCH-L1 dimer and hence may explain the reduced dimerization-dependent ligase activity of UCH-L1-S18Y in comparison to UCH-L1-WT. *Acknowledgements: Research Grant Council of Hong Kong (GRF 7755/08M and 7765/09M)*

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SERINE PHOSPHORYLATION OF TrkB BY Cdk5 IS REQUIRED FOR ACTIVITY-DEPENDENT STRUCTURAL PLASTICITY AND SPATIAL MEMORY

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The neurotrophin brain-derived neurotrophic factor (BDNF) and its receptor TrkB participate in diverse neuronal functions, including activity-dependent synaptic plasticity that is crucial for learning and memory. Besides auto-phosphorylation at tyrosine residues, TrkB also undergoes serine phosphorylation at S478 upon binding to BDNF by the serine/threonine kinase cyclin-dependent kinase 5 (Cdk5). However, the in vivo function of this serine phosphorylation remains elusive. We have generated knock-in mice lacking this serine phosphorylation (trkBS478A/S478A), and here we report that the TrkB phosphorylation-deficient mice display impaired spatial memory and compromised hippocampal long-term potentiation (LTP). S478 phosphorylation of TrkB regulates its interaction with the Rac1 specific guanine nucleotide exchange factor (GEF) TIAM1, leading to activation of Rac1 and phosphorylation of S6 ribosomal protein during activity-dependent dendritic spine remodeling. These findings reveal the importance of Cdk5-mediated S478 phosphorylation of TrkB in activity-dependent structural plasticity that is crucial for LTP and spatial memory formation.

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THE INTRINSIC DYNAMICS DRIVEN BY DYNAMICAL SYNAPSES COULD COMPENSATE DELAYS IN NEURAL PROCESSES

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Delays can be found in the neural system. At the subcellular level, it takes time for the action potential to travel form the soma to the axon terminal. For the post-synaptic neurons, the change in the membrane potential for a spike also takes time. So, neurons cannot react immediately to changes in external stimuli. These delays can be accumulated to produce significant effects. However, in many real-life situations, timely reactions of the nervous system are required. For moving objects, the neural system should be able to predict the future position of the object based on the current motion. This behavior is known as anticipation. Recently, in a model of continuous attractor neural networks (CANNs), we found that short-time synaptic depression (STD) can increase the mobility of attractor states that are used to encode continuous information. This enhances the performance of tracking changes of external stimuli. In this work, we propose an anticipatory mechanism based on this intrinsic dynamics of continuous attractor neural network with short-term synaptic depression. With different levels of STD, different degrees of anticipation can be obtained. Also, there are particular levels of STD such that the CANN can achieve zero-delay tracking. Moreover, if the speed of the external stimulus is close to the intrinsic speed of the attractor states of the system, the delay of the network response will be effectively independent of the strength of the stimulus. To our knowledge, this phenomenon has not been reported in the literature. This work is partially supported by the Research Grants Council of Hong Kong *(grant nos. 604008 and*

605010).

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AN INVESTIGATION INTO THE ROLE OF AGS3 IN OPIATE TOLERANCE AND DEPENDENCY

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The receptor-independent activator of G protein signaling protein 3 (AGS3) has previously been implicated in the establishment and maintenance of opiate tolerance and dependency. Increased levels of AGS3 expression in the nucleus accumbens are found to associate with withdrawal after chronic administration of cocaine or morphine. Moreover, siRNA knockdown of AGS3 expression has been shown to restore normal signaling and eliminate withdrawal symptoms and drug-seeking behaviors. AGS3 is a 650-amino acid protein capable of binding up to four Gai-GDP subunits. It acts as a guanine-nucleotide dissociation inhibitor (GDI) by stabilizing Gai-GDP, preventing its re-association with the Gbg subunits and thus lead to three possible outcomes: (1) Activation of Gbg signaling by AGS3 competing against Gbg for Gai-GDP from the pool of heterotrimeric complexes and triggering release of Gbg subunits for downstream signals; or (2) AGS3/Gai-GDP complex acting as a signaling molecule having specific downstream effectors; or (3) AGS/Gai-GDP complex acting as a substrate for GEF to generate Gai-GTP. In the present study we have shown that AGS3/Gai-GDP complex does not act as a signalling molecule by itself and may not be a substrate of GEF in HEK293 system. Moreover, our preliminary data showed that AGS3 does not activate Gbg signalling. Possible interaction between Ric8A, a GEF, and AGS3 is being pursued and co-immunoprecipitation assays have indicated strong interactions between them. Supported by the Research Grants Council of Hong Kong (661807).

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NANO-STRUCTURED PLATFORM FOR HIGHLY SELECTIVE, SENSITIVE AND QUANTITATIVE ELECTROCHEMICAL DETECTION OF CARBON MONOXIDE (CO) IN BIO-MIMETIC MEDIA

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Carbon monoxide (CO) as a byproduct of heme catabolism and lipid oxidation has been recognized for many years, but its role as an essential signaling molecule was only discovered recently after that's of nitric oxide (NO). Quite diverse physiological functions of CO have been identified, including anti-inflammation, vasodilatation and cytoprotection. The endogenously generated CO is also involved in long term memory formation by activation of NMDA receptor. On the other hand, CO is also known to be a toxin , acting as a silent killer by binding much more strongly than O_2 to hemoglobin and cytochrome oxidase. The measurement of CO concentration becomes very important for the elucidation of its functional mechanism and regulation pathways, as well as for assessing and exploring its therapeutic applications. As such, it is highly desirable to have methodologies and techniques for its detection in a selective, sensitive and quantitative manner and in the presence of its interfering chemical species.

In this report, we describe a method developed recently in our group or the detection of CO in aqueous solution. The method was built on the similar nano-structured electrochemical platform that we have developed for the detection of H_2O_2 and glucose but with one important difference: we have chosen a nano-structured electro-active pseudo-catalyst (electrochemical mediator), Cu_2O, for the detection of CO because CO is an electronically very inert molecule toward either catalytic oxidation or reduction in aqueous medium and at ambient condition. By selecting Cu_2O as a pseudo-catalyst (electrochemical mediator), we employed a non-redox reaction, yet electrochemical active approach for the detection of CO in aqueous solution selectively and quantitatively. This approach has an intrinsic advantage of immune to many interfering species. The pros and cons, as well as our current effort in improving the sensitivity of our method will be presented.

Acknowledgement: We acknowledge the support of this project by RGC-HK, WMINST-HKUST, RPC-HKUST, and BIEN PG Program-HKUST

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NANO-STRUCTURED PLATFORM FOR HIGHLY SELECTVE, SENSITIVE AND QUANTITATIVE ELCTROCHEMICAL DETECTION OF HYDROGEN SULPHIDE (H2S) IN BIO-MIMETIC MEDIA

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Hydrogen sulphide (H_2S) is famous for its unpleasant rotten egg smell and was traditionally considered as a toxic gas. After the discovery that nitric oxide (NO) can act as a signaling molecule to control a variety of biological processes , similar role of H_2S has also been revealed. H_2S is endogenously generated in many cell types inside human body and has been associated with many important physiological functions, including anti- inflammation, vasodilatation, cardioprotection and long term memory formation among many others. Adding to this list is a recent study showing that a mouse entered hibernation mode after applying of H_2S [1]. Similar to other small redox-active messengers, H_2S has shown a double-faced and double-edged biological identity, being both a toxin and a signaling messenger depending on the concentration and location of its endogenous production. While the demand for its in situ detection in a selective, quantitative, and sensitive manner is increasing, any effort for this goal has to overcome a serious challenge posed by the interference of many co-existing thiol-containing species such as cysteine, methionine and glutathione among others.

We report here a very promising method for the highly selective, sensitive and quantitative detection of H_2S in aqueous solution and in the presence of the most common interfering species such as cysteine, methionine, and glutathione. Our method was built on the nano-structured electrochemical platform we developed for the detection of H2O2 and glucose, but with a selection of nano-structured gold (Au) catalyst uniquely well-suited for thiol-containing compound such as H_2S. Special effort was spent on solving the interfering problem caused by cysteine, methionine and glutathione.

Acknowledgement: We acknowledge the support of this project by RGC-HK, WMINST-HKUST, RPC-HKUST, and BIEN PG Program–HKUST.

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MOUSE SACRAL NEURAL CREST CELL MIGRATION *EX VIVO* AND *IN VITRO*

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The enteric nervous system is derived from neural crest cells (NCCs) in embryos and all enteric ganglia in the distal colon originate from the neural crest at both vagal and sacral levels. During the early embryonic gut development, vagal NCCs migrate caudally toward the hindgut (embryonic distal colon) while sacral NCCs progress rostrally toward the cecum. These two populations of NCCs meet in the hindgut to form a complete neuronal network in the colon. In this study, we aimed to examine the dynamic migratory behaviours of sacral NCCs *ex vivo* and *in vitro* using confocal live cell imaging. When hindgut segments were explanted and cultured *ex vivo*, sacral NCCs migrated individually along nerve fibres extending from the pelvic ganglia while most of vagal NCCs moved in chains within the hindgut. When a sacral NCC met a vagal NCC on a nerve fibre, they collided, pushed toward each other and then intermingled to form cellular networks with much greater contribution from vagal than sacral NCCs. Similarly, *in vitro* culture of pelvic ganglia showed that sacral NCCs also migrated along nerve fibres extending from the ganglia. If

the extension of fibres from the pelvic ganglia was perturbed by wheat germ agglutinin, the distribution of sacral NCCs along nerve fibres was also altered. If the fibre extension was delayed in a proliferation medium, many sacral NCCs were found outside the fibres, indicating that nerve fibres were permissive but not necessary for sacral NCC migration under this *in vitro* condition. In conclusion, our results demonstrate that sacral NCCs exhibit migratory behaviours which are distinct from those of vagal NCCs.
Acknowledgements: This work was supported by a grant from the Research Grants Council of the Hong Kong Special Administrative Region, China (Project no. CUHK461808).

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NM23-H1, BUT NOT NM23-H2, INHIBITS ONCOGENE-INDUCED NEOPLASTIC TRANSFORMATION

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G proteins (guanine nucleotide-binding proteins) are a family of proteins playing a key role in transmitting chemical signals from outside the cell into intracellular events. G proteins can be turned on by GEF (guanine nucleotide exchange factor) that exchanges GDP for GTP on the Gα subunit. On the other hand, G proteins can be turned off when the Gα subunit eventually hydrolyzes the attached GTP to GDP by its inherent enzymatic activity, a process which can be accelerated by GAPs (GTPase-activating proteins). Recently, members of a novel protein superfamily, known as "regulators of G-protein signalling" (RGS), have been identified as potent GAPs for Gα subunits. RGS19 is one member of the RGS family. Previous studies in our lab have revealed that RGS19 can inhibit RasGV-induced neoplastic transformation (RasGV is the constitutively active form of Ras), but the mechanism remains unknown. Interestingly, over-expression of RGS19 was able to up-regulate Nm23 (non-metastatic 23) which acts as a nucleoside diphosphate kinase. Given that Nm23-H1 is a well-known tumor metastasis suppressor and is involved in the inhibition of colonization and motility, its up-regulation may well explain the inhibitory actions of RGS19 on oncogenic Ras-induced neoplastic transformation. Likewise, Nm23-H2 acts as a tumor metastasis suppressor and is capable of modulating the transcription of c-myc gene. Hence, the present study examined the effect of overexpression of Nm23-H1 and Nm23-H2 on oncogene-induced neoplastic transformation in NIH 3T3 fibroblasts. Our data revealed that Nm23-H1, but not Nm23-H2, can suppress neoplastic transformation and tumor formation in nude mice. They are also reports indicating that Nm23 plays important roles in nervous system, including influencing neurite outgrowth, neuronal proliferation, differentiation and apoptosis. Supported by the Research Grants Council of Hong Kong (663110).

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STRUCTURE-ACTIVITY RELATIONSHIPS OF NOVEL MELATONIN MT2 SUBTYPE-SELECTIVE LIGANDS WITH NON-INDOLEAMINE SCAFFOLDS

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The notion of melatoninergics as promising chronobiotics for treating various circadian rhythm sleep disorders is well accepted and has resulted in the generation of a range of bioisosteres of melatonin. However, the area of subtype-selective therapeutic melatoninergics has not been thoroughly addressed and many of the synthetic melatoninergics act on both subtypes of melatonin receptors with minimal selectivity. A series of novel compounds were designed and synthesized based on non-indoleamine heterocyclic scaffold and their binding affinities towards human melatonin MT_1 and MT_2 receptors were evaluated. Heterocyclic compounds bearing a phenyl group at their 4 position, displayed higher binding affinities towards MT_2 than MT_1 receptors, indicating the importance of a phenyl substituent as a consistent motif to achieve MT_2 selectivity. Subtype selectivity of the compounds was further enhanced by extending the acetamide side-chain to n-propyl group, which had shown to increase the MT_2, but maintained the MT_1 affinities. Most of the test compounds were potent MT_2-selective agonists as revealed in receptor-mediated intracellular Ca^{2+} mobilization and phosphorylation of extracellular signal-regulated protein kinases, in line with their affinities derived from binding assay. Intriguingly, compounds bearing a

phenyl substituent on their acetamide side-chain suppressed melatonin-mediated receptor activation in a MT_2-selective manner. Increasing concentration of the potential antagonist markedly attenuated the maximal response of melatonin and shifted the melatonin dose-response curve. The development of subtype-selective melatoninergics may pinpoint the distinct roles of MT_1 and MT_2 receptors in the regulation of circadian rhythmicity and result in a safer medicine with more favorable pharmacological profiles.

Supported in part by grants from RGC and UGC of Hong Kong (HKUST661808 and AoE/B-15/01) to YHW.

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THE FUNCTION OF TYROSINE PHOSPHATASE IN THE DEVELOPMENT OF NEUROMUSCULAR JUNCTION

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The neuromuscular junction (NMJ) is a synapse that develops between a motor neuron and a muscle fiber. During NMJ development, acetylcholine receptors (AChRs) are clustered to a high density on the postsynaptic membrane. Before innervation, AChRs spontaneously form clusters, termed pre-patterned clusters, on muscle membrane. When motor axons course along muscle, neural agrin activates muscle-specific kinase (MuSK) to disperse pre-patterned clusters ("global effect") and to induce new clusters at postsynaptic sites ("local effect"). Our previous studies have suggested that the global effect is mediated by the protein tyrosine phosphatase (PTP). In this study, we aim at investigating the function of PTP in the local effect of AChR clustering. HB-GAM coated beads were used to induce AChR clustering in cultured Xenopus embryonic muscle cells as a mimicry to innervation and bpV (potassium bisperoxo [1,10-phenanthroline] oxovanadate; phen), a novel PTP inhibitor, was used to examine its involvement. AChRs form highly localized clusters with discrete boundary in response to bead stimulation. Treating cells with bpV caused pronounced spread of AChR clustering as evidenced by an increase in the cluster size and a decrease in AChR density. Besides beads, AChR clustering induced by spinal neurons was also studied. Receptors clusters at nerve-muscle contacts also became broadened beyond the subsynaptic area following bpV treatment of newly established NMJ. However, pre-treatment of bpV before nerve-muscle contact significantly reduced synapse formation. Together with our previous data, these results suggest that in addition to kinase, PTP is essential for the formation of high-density AChR clusters at the NMJ and one of its roles is in defining the zone of kinase activity to generate a sharp boundary of postsynaptic specialization. (Supported by Hong Kong RGC GRF grant 662311)

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CAMSAP1L1 IS A POTENTIAL EPILEPSY GENE IN THE CHINESE POPULATION

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Abstract Purpose: In a genome-wide association study of Chinese epilepsy and control subjects, we found association of the CAMSAP1L1 gene with epilepsy. Because very little is known about the protein, we sought to explore its localization and function. Methods: We performed Western blotting of homogenates of human and mouse brain and human neuroblastoma SH-SY5Y cells, and immunohistochemistry of fixed brain sections and SH-SY5Y cells. Results: Two antibodies (161A and 162A) map to different epitopes of human CAMSAP1L1 and stained a band at the expected molecular weight of approximately 170 kDa in humans. Only 162A also stained this band in mouse samples, as predicted by conservation of the epitope sequences in mice. CAMSAP1L1 immunoreactivity appeared in neurons in human hippocampal sections and in the cytosol and neurites of SH-SY5Y cells. Proteins of the CAMSAP family have a conserved C-terminal region called the CKK domain, which is predicted to bind to microtubules and may block microtubule function to inhibit neurite outgrowth. Double immunofluorescence staining in SH-SY5Y cells showed partial colocalization of CAMSAP1L1 with class III beta-tubulin. Conclusion: A genetic variant of

CAMSAP1L1 may be associated with epilepsy. CAMSAP1L1 is expressed in neurons and may bind microtubules, suggesting a potential role in epileptogenesis via an effect on the extension of neurites.

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MODULATION OF SYNAPTIC TRANSMISSION AND LONG-TERM SYNAPTIC PLASTICITY BY EXTRACELLULAR IRON IN THE RAT HIPPOCAMPUS

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Iron accumulation in the brain has been implicated in the pathogenesis of a number of neurodegenerative disorders, primarily attributable to its role in increasing oxidative stress, leading to neuronal vulnerability. However, the exact effect of iron in brain function, especially at early stages of accumulation, is unknown. In this study, we investigate whether acute iron accumulation would affect synaptic transmission and long-term potentiation (LTP) in the hippocampus, a region known to be of critical importance in learning and memory. Slices of the hippocampus were prepared from adult Sprague-Dawley rats and maintained in vitro. Field excitatory postsynaptic potential (fEPSP) recordings were performed on the CA3-CA1 pathway. Basal synaptic transmission was not affected by lower doses of 0.3μM and 40μM ferric ammonium citrate (FAC), but was significantly impaired at high doses of 100μM and 500μM. Paired pulse ratio was not affected by any concentrations of FAC, indicating a postsynaptic origin of impairment. LTP was reduced significantly at 40μM FAC and above, but had no change at 0.3μM. To elucidate the underlying mechanisms of these experiments, we performed whole-cell patch-clamp recordings on CA1 pyramidal cells with stimulation applied to the Schaffer collateral, and AMPA-receptor and NMDA-receptor mediated EPSCs were obtained by maintaining the membrane potential at -60mV and +40mV respectively. Both AMPA and NMDA receptor currents were significantly reduced in amplitude after administration of 100μM FAC, and the effect was long-lasting. Taken together, we obtained evidence that extracellular ferric iron could cause acute impairment in basal synaptic transmission and LTP in the hippocampus by inhibiting postsynaptic receptors of both AMPA and NMDA types. This potentially adulterates the neuronal functions in cognitive processes even in the absence of neuronal death. The results also suggest that a brief rise in iron is likely to exert a long-lasting effect on brain function.

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THE NEUROTOXIC EFFECTS OF INTRANEURALLY INJECTED RIBOSOME-INACTIVATING PROTEINS (RIPS) ON DORSAL ROOT GANGLION NEURONS

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The neurotoxic effects of intraneurally injected ribosome-inactivating proteins (RIPs) on dorsal root ganglion neurons. WZ Shen, O Sha*, J Zhang, XL Feng, T Zhao, WH Kwong*. Abstract: Objective. To investigate the different peripheral neuronal toxicity of two types of RIPs, including type I ricin A chain (RTA) and a-trichosanthin (TCS), and type II ricin 120. Methods. TCS, RTA and ricin 120 were separately injected into the rat sciatic nerve. Saline was used alone and separately as control solutions. The paraffin sections of dorsal root ganglia were prepared and the sensory neurons were investigated and counted for quantity evaluation. Results. All RIPs were taken up and transported by peripheral axons. TCS at a dose of 1 nmol killed only one-third of the sensory neurons of the injected nerve. RTA at a dose of 0.22 nmol killed about two-thirds and ricin 120 at a dose of 0.63 nmol eliminated nearly all sensory neurons. The loss of neurons was permanent. Conclusion. RIPs are retrogradely transported by axons of the injected nerve. Type I RIPs, RTA and TCS, show a selective neurotoxicity on different types of neurons. Hence type I RIPs are useful in producing neural lesion in research, and this use may also be of applicational value in treating chronic spasticity, hyperalgesia, and pain. (NSFC Grant: No. 81171154)

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DRAGON'S BLOOD AND ITS COMPONENTS INHIBIT THE ACTIVITY OF ACID-SENSING ION CHANNELS IN RAT SENSORY NEURONS

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Tissue acidosis, which occurs in a wide range of pathological states, has an important role in pathogenic of pain. It is generally believed that acid-sensing ion channels (ASIC) mediate the great part of acid-induced nociception in mammals. Dragon's blood (DB), a well-known traditional herbal medicine, is clinically effective for relieving pain. Here we explored the effects of Dragon's blood and its three components: loureirin B (LB), cochinchinemin A (CA) and cochinchinemin B (CB) on inward ASIC currents which were elicited by extracellular acidosis in acutely isolated rat dorsal root ganglion (DRG) neurons. We found that Dragon's blood and its three components inhibited ASIC currents, and combinations of two or all three components produced additive or synergistic inhibitory effects on ASIC currents. These results indicated the Dragon's blood and its components can block ASIC currents in rat DRG neurons and these effects may account for analgesic effect of Dragon's blood. Therefore, it may provide a potential approach for developing new pain therapy by manipulating the combinations of those components.

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UNDERSTANDING THE ROLE OF Cdk5 IN ACTIVITY-INDUCED GENE EXPRESSION DURING DENDRITE DEVELOPMENT

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The formation and remodeling of neuronal connections during nervous system development is a highly dynamic process and is influenced by neuronal activity. The regulation of gene expression by neuronal activity is crucial to fine-tune neuronal connections, but how neuronal activity sends signals to the nucleus to regulate gene transcription remains unclear. Our laboratory has demonstrated that the proline-directed serine/threonine kinase Cdk5 is crucial for the growth of dendrites in cultured hippocampal neurons. Interestingly, we found that Cdk5 and its activators p35 and p39 were localized not only in the cytoplasm but also in the nucleus in neurons. These observations prompt us to hypothesize that Cdk5 might have an important role in the nucleus by regulating activity-dependent gene transcription during dendrite development. Consistent with the hypothesis, the defects in dendritic growth upon knockdown of Cdk5 in cultured hippocampal neurons can be rescued by wild-type Cdk5, but not the nuclear-localization deficient mutant, indicating that the nuclear localization of Cdk5 is required for dendrite development. Biochemical fractionation revealed the increased accumulation of Cdk5 in the nucleus after neuronal depolarization. In addition, the depolarization-induced mRNA expression of the neurotrophin bdnf was greatly attenuated by the Cdk5 inhibitor roscovitine. Finally, the induction of dendritic growth after neuronal depolarization was abolished in the presence of roscovitine, as well as in Cdk5 knockout hippocampal neurons. Together these observations suggest a novel function of Cdk5 in activity-dependent dendritic growth by regulating gene transcription in the nucleus.

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SPATIAL CODING PROPERTY OF DORSOMEDIAL CELL COLUMN NEURONS IN THE VESTIBULO-OLIVARY PATHWAY

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The inferior olivary complex is an important relay of vestibular information to the cerebellum. Experiments were conducted in adult Sprague-Dawley rats to characterize the spatial coding features of neurons in dorsomedial cell column (DMCC), an inferior olive subnucleus that is implicated to process otolithic signals. Connection between vestibular nucleus and DMCC was identified by combined

retrograde tracing (Fluorogold, FG) and *c-fos* expression, which has been used as a means to document neurons functionally activated by natural otolithic stimulation. FG-labeled neurons were mainly found in the spinal vestibular nucleus (VN) while a smaller number was found in the medial VN and prepositus hypoglossal nucleus. Fos-positive DMCC neurons were observed with horizontal linear acceleration along the transverse direction but not the antero-posterior direction. Control animals, namely labyrinthectomized rats subjected to linear acceleration and normal rats that remained stationary, showed only a few sporadically scattered Fos-positive neurons. That FG-labeled VN neurons showed *c-fos* expression indicates direct projection of spatial signals from VN to DMCC. The directional responsiveness of otolith-related DMCC neurons was confirmed with in vivo extracellular recoding. The best response orientations of these neurons pointed only in the left-side-down quadrant or right-side-down quadrant, but not in the head-down quadrant and head-up-quadrant. Taken together, our results indicate that DMCC neurons receive otolithic inputs from the VN and encode translational head movements along the transverse direction on the horizontal plane. [Acknowledgement: RGC Grant 761710M]

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ACIDOSIS INDUCED INJURY IN CEREBRAL ISCHEMIA: THE ROLE OF ASIC1A

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Objective of work: A dramatic increase in intracellular calcium acting through various pathways has been held responsible for neuronal cell death in cerebral stroke. Earlier this calcium overload was considered to be mainly glutamate dependent but the failure of glutamate antagonists in clinical trials suggested the existence of glutamate independent mechanisms contributing to neuronal injury. Recently Acid Sensing Ion Channels (ASIC`s), which are activated following acidosis in ischemic brain, were found to promote neuronal Ca^{++} influx and subsequent neuronal death in stroke. The present study was done to investigate the neuroprotective potential of ASIC inhibition in cerebral ischemia/reperfusion injury.

Methodology: Focal cerebral ischemia was induced in male S.D. rats (250 ± 20g) by occlusion of middle cerebral artery. ASIC inhibitor, Flurbiprofen (10mg/kg, i.p.) was administered prior and post induction of ischemia to assess its therapeutic window. Neurological deficit, brain infarction and SBDPs levels were measured to assess the neuroprotection conferred through ASIC inhibition. Moreover western blotting was done to analyze the expression of ASIC in affected brain region at different time points of ischemia/reperfusion injury

Results and Discussion: The flurbiprofen treatment, thirty minutes prior to ischemia and four hour post reperfusion, afforded significant neuroprotection from ischemic injury as evidenced by reduction in cerebral infarct volume and neurobehavioral deficit. It also reduced the proteolytic products (SBDPs) caused by ischemic activation of calcium dependant protease calpain. Moreover, an increase in ASIC expression at late time points of I/R injury was observed in ischemic rat brain regions. These results indicate the profound involvement of ASIC's in cerebral stroke and warrant for more studies to be done.

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EFFECTS OF 5-HYDROEXYTRPTAMINE ON THE FIRING OF SUBTHALAMIC NEURONS IN NORMAL AND PARKINSONIAN RATS

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Objective: The subthalamic nucleus (STN) plays a critical role in movement regulation. 5-hydroxytrptamine (5-HT) has been reported to play an important role in the basal ganglia. Anatomical and morphological studies indicated that STN receives serotonergic innervation from raphe nuclei and expresses several serotonergic receptor subtypes. The present study is to further investigate the effects of 5-HT in STN of normal and parkinsonian rats.

Methods: *In vivo* extracellular recording were performed in our studies. Parkinson's disease rat model

was constructed by microinjection of 6-OHDA into lateral medial forebrain bundle.

Results: (1) The basal firing rate and pattern in STN of 6-OHDA parkinsonian rats were not significantly different from that of normal rats. (2) In normal rats, micro-pressure ejection of 5-HT (0.1 mM) increased the spontaneous firing rate from 11.6±2.9 Hz to 14.7±3.5 Hz in 7 out of 22 STN neurons with the average increase of 30.85±3.56 % ($P<0.01$), and decreased the firing from 5.4±1.3 Hz to 3.5±1.2 Hz in 3 out of 22 neurons ($P<0.05$). Furthermore, in 6-OHDA parkinsonian rats, 5-HT increased the firing rate of STN neurons by 22.40±1.0 % in 5 out of 10 STN neurons (basal: 14.0±4.8 Hz, 5-HT: 17.1±5.8 Hz, $P<0.05$), and decreased the firing only in one STN neuron.

Conclusion: Based on the electrophysiological actions of 5-HT in subthalamic nucleus, we demonstrated that 5-HT modulated the firing activities of STN neurons in both normal and parkinsonian rats.

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NEUROPROTECTIVE EFFECT OF SESAMOL AND 7-NI IN UNPREDICTABLE CHRONIC STRESS-INDUCED DEPRESSIVE-LIKE BEHAVIOUR: ROLE OF NITRIC OXIDE PATHWAY

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There is some strong evidence of nitric oxide (NO) as an intercellular messenger in central nervous system (CNS). NO is synthesized from L-arginine by nitric oxide synthase (NOS), as a response to activation of N-methyl-D-aspartate (NMDA) receptors by excitatory amino acids. In preclinical models, NOS inhibitors and NMDA receptor antagonists also produce antidepressant-like actions. 7-Nitroindazole (7-NI) is a selective inhibitor of neuronal NOS in CNS. The present study was designed to evaluate the role of nitric oxide in the antidepressant action of sesamol and to evaluate the effect of 7-NI in unpredictable chronic mild stress (UCMS)-induced depression. In this study, the animals were subjected to different stress paradigms daily for a period of 21 days to induce depressive-like behaviour. The immobility period, locomotor activity, memory acquisition and retention were evaluated on 21st day. Results showed that pre-treatment of L-arginine significantly reversed the protective effect of sesamol (8 mg/kg) on chronic stressinduced behavioural (immobility period) and biochemical (lipid peroxidation & nitrite levels; glutathione levels, superoxide dismutase & catalase activities) in stressed mice. Furthermore, L-NAME (10 mg/kg) pre-treatment with a sub effective dose of sesamol (4 mg/kg) significantly potentiated the protective effect of sesamol. Treatment with 7-NI also reversed UCMS-induced behavioural and biochemical in stressed mice. Hence, the present study demonstrated that nitric oxide modulated signalling might be involved in antidepressant like effects of sesamol in stressed mice and the protective role of 7-NI (nNOS inhibitor) in UCMS-induced depression in mice was also confirmed.

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QUERCETIN LOADED NANOFIBER PATCH IMPROVES THE FUNCTION OF NERVE AND OXIDATIVE DAMAGE IN EXPERIMENTAL DIABETIC NEUROPATHY RAT

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Introduction: Diabetic neuropathy is one of the challenges in this decade. Recent findings showed that oxidative stress played a crucial role on the pathophysiology of this condition. Therefore, this study was set up to determine whether quercetin loaded nanofiber patch could enhance nerve recovery in experimental diabetic neuropathy with very low concentration.

Materials & Methods: Male Wiatar rats, weighing 200-250, were induced diabetes mellitus using sterptozotocin. The rats were induced mononeuropathy at sciatic nerve by crushing. The nerve was crushed

for 30 sec using hemostatic forceps at mid thigh position of right leg. Then, they were exposed to quercein loaded nanofiber patch ($1cm^2$) at concentration of 5 and 10% for 21 days. The dressing patch was changed every day. The assessment of motor function was performed using DeMedinacelli method and muscle power while the sensory function was performed using foot-withdrawal reflex test every 3 days throughout the experimental period. At the end of experiment, the oxidative stress markers including MDA level and the activities of SOD and CAT were also determined.

Results and discussion: 5%Quercetin loaded nanofiber patch significantly reversed the decrease sensitivity to foot withdrawal reflex test. No significant changes were observed in sciatic function index and muscle power. In addition, rats subjected to 5%qurcetin patch enhanced SOD activity but decreased MDA level. Therefore, our data suggested that quercetin effectively decreased oxidative damage in the sciatic nerve which in turn reversed the functional impairment in experimental diabetic neuropathy.

Conclusion: Quercetin loaded nanofiber patch is the potential candidate for diabetic neuropathy treatment which can provide the effectiveness at the concentration less than oral route intervention more than 124 times and this may also reduce risk of adverse effect. However, further research in clinical trial and the quality control of the products are still essential. *Acknowledgement: This work has partially been supported by the National Nanotechnology Center (NANOTEC), NSTDA, Ministry of Science and Technology, Thailand through its program of Center of Excellence Network and the Integrative Complimentary Alternative Medicine Research Group, Khon Kaen University, Thailand.*

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EFFECT ON IONIC GABA RECEPTOR IN RAT CEREBELLUM GRANULE NEURONS INDUCED BY 50 Hz ELECTROMAGNETIC FIELD.

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Introduction: Nowadays, 50 Hz electromagnetic field (EMF) exists in almost every aspect of modern life, especially near the high-voltage wire. Lots of research about non-ionic radiation has been conducted. We used self-made electromagnetic exposure system to study electromagnetic effect of 50 Hz on neuron system. Rat cerebellar granule neurons (CGN) were used as model cell, which are excitatory interneurons in cerebellum and form the important parallel fibers. Ionic GABA receptor can induce chlorine current, when acted by its ligands.

Methods: Cell culture, patch-clamp whole cell recording, electromagnetic exposure, western-blot.

Results: Previously, we find that 50 Hz electromagnetic field can hardly make any influence on potassium current of rat cerebellar granule neurons. But in this research, results showed that chlorine current of rat cerebellar granule neurons induced by GABA increased after 1 hour exposed by 1 mT 50 Hz electromagnetic field. And dose-dependant effect also exists. But the mechanism of how electromagnetic field influent ionic GABA receptor and the intercellular signal pathway relevant to these phenomena were still under research.

Conclusion: 50 Hz electromagnetic field can change the amplitude of chlorine current of rat cerebellar granule neurons induced by GABA. And mechanism of these phenomena need further research.

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POST-STROKE NEURAL ACTIVITIES USING ELECTROCORTICOGRAM (ECOG) IN A RAT MODEL OF FOCAL ISCHEMIA

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Monitoring the ischemic penumbra is important for treatment and outcome prediction after stroke. Electrocorticogram (ECoG) is a direct, convenient brain monitoring method by recording the electrical signals from cortical surface. However, the application of ECoG in experimental brain ischemia is not well studied. The objective of this study is to investigate the ECoG activity in a rat model of focal ischemia from the acute phase to the chronic phase.

In this study, focal cerebral ischemia was induced by intraluminal suture middle cerebral artery occlusion (MCAo). Throughout the acute phase, subacute phase and chronic phase, ECoG were recorded from the

bilateral sensorimotor cortex using stainless steel electrodes. The alpha-to-delta ratios (ADRs) and peak power variability were calculated from the ECoG recordings. The rat's motor functions were measured by De Ryck's test and beam walking test. Brain Infarct was revealed by 2, 3, 5-triphenyltetrazolium chloride (TTC) staining.

Quantitative ECoG analysis showed dominant delta activity in the acute phase and suppression of alpha/beta activity in the subacute phase. Alpha/beta variability decreased during subacute phase and fully recovered in the chronic phase. ADRs were below 50% of the pre-stroke level at both acute and subacute phases, and the improvement in ADRs was highly correlated with sensorimotor recovery ($r = 0.9895$, $p<0.05$). In addition, TTC stained brain slices confirmed that striatum infarct completed within 24 hours and the cortical infarct expanded until 72 hours.

Altogether, this study demonstrated that ECoG provided information on the stroke pathophysiology in a rat model of focal ischemia from acute phase to chronic phase. Post-stroke functional recovery is closely related to the restoration of neural activity in the penumbra. Quantitative ECoG parameters, such as ADR and peak power variability, have the potential to be used in stroke diagnosis and prognosis.

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EVIDENCE FOR AN ANTIHHYPERALGESIC EFFECT OF FLAVONOID FRACTION OF *VITAS VINIFERA* IN CCI-INDUCED NEUROPATHY IN RAT

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Objective: To investigate the protective effects of flavonoid fraction of *Vitis vinifera* (FFVV) on the CCI-induced pain and accompanied depression in rats. Material and methods: *Vitis venifera (VV)* fruits were authenticated from botanical survey of India, Pune and flavonoid fraction was isolated from the fruits. Male Wistar rats (180-200g) were operated under pentobarbital (60mg/kg i.p.) anesthesia for chronic constriction injury (CCI). Animals were inspected every day and were tested on 28th day. Saline (3ml/kg), FFVV (50, 100 and 150 mg/kg) gabapentin (100 mg/kg) were administered by *intraperitonially* from 21st - 28th day after surgery & subjected to the following tests: 1) Randall Selitto analgesiometer; 2) Thermal hyperalgesia and allodynia; 3) Actophotometer; 4) Rotarod test; 5) Forced swimming test (FST).

Statistical analysis: The results were expressed as means ± SEM and analyzed by using t-test and ANOVA. $p<0.05$ was considered as significant.

Result and Discussion: CCI induced animals displayed significant hyperalgesia, allydonia ($p<0.05$) in hot and cold plate and likewise demonstrated significant increased in the time of immobility (depression) in FST ($p<0.05$) when compared with sham operated animals. As the time of swimming was unchanged, immobility was increased at the expense of climbing behaviour ($P<0.005$). There was no difference seen in ambulation and motor activity. FFVV (150 & 100 mg/kg) & Gabapentin (100 mg/kg) significantly attenuated the hyperalgesia, allydonia ($p<0.05$) and decreased the time of immobility ($p<0.05$). FFVV 50 mg/kg has not shown statistically significant results.

Conclusion: In the present study, animals subjected to CCI displayed the chronic neuropathic pain & depression behavior (increased immobility in FST) which mimics the clinical condition. FFVV (100 and 150 mg/kg *i.p.*) shown potential to attenuate pain—depression syndrome in rats.

P95

OCT4 VIRAL VECTOR DELIVERY TO MOUSE BRAIN CAN INCREASE PLURIPOTENCY MARKERS' EXPRESSION AND VALPROIC ACID ENHANCES ITS EFFECT.

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Introduction: There are several reports of somatic cell reprogramming into pluripotent stem cells (iPSCs) with a combination of four transcription factors, Oct4/Sox2/Klf4/c-Myc. Interestingly, NSCs endogenously express Sox2, c-Myc, and Klf4, so they were reprogrammed into iPSCs just with Oct4 although the

efficiency was low. Besides, some chemical compounds such as valproic acid can improve the efficiency of reprogramming. Increasing neural stem cell number and their potentials could be useful in brain repair.
Methods: Oct4 lentiviral vector was injected into the lateral ventricle of C57/BL6 mice brain followed by administration of doxycycline (3 µg/ml) for 5 consequent days. Valproic acid was also administered via oral gavage in 100 µl twice daily. Animals were decapitated at day 7 and total RNA was extracted from the tissue collected from the rims of lateral ventricles. After cDNA synthesis, real time PCR performed to analyze the expression of Oct4, Sox2, Nanog, Klf4, c-Myc, Sox1and Pax6. Gene expression levels were normalized to GAPDH as an internal standard. Moreover, some of the brains were fixed and processed for analyzing expression of Oct4 and Nanog proteins in addition to SSEA1 using immunohistochemistry.
Results: Endogenous expression of Klf4, Nanog, c-Myc, Sox1 and Pax6 mRNAs were increased 7 days after administration of Oct4 lentiviral vector, but VPA administration can improve Klf4, Nanog and c-Myc expression significantly. In addition, increased level of SSEA1 was detected in the brain sections of the group which got valproic acid in addition to Oct4 lentiviral vector.
Conclusion: Exogenous expression of Oct4 vector can induce cells reside in the rim of lateral ventricles to express genes activated in the pluripotency state and valproic acid can enhance its effect. This can improve neural stem cell's potencies in SVZ to repair brain degenerations more efficiently.

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ALZHEIMER AND ATP SENSITIVE CA2+ ACTIVATED POTASSIUM CHANNEL IN BRAIN MITOCHONDRIAL INNER MEMBER

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Alzheimer's disease is a progressive neurodegenerative disorder, characterized clinically by the impairment of cognitive functions and changes in behavior and personality. Recent evidence suggests that mitochondrial channel plays an important role in memory disorders. Accordingly, we investigated the biophysical properties of ca^{2+}-activated potassium channels (mito BK) in brain mitochondrial inner membrane in rat with Alzheimer's disease.

We induced Alzheimer to male wistar rats (220- 250g) by intracerebroventricular injection of amyloid beta 1-42(4µg/µl). After two weeks, brain mitochondrial inner membranes were extracted. Vesicles incorporated into bilayer lipid membranes and single potassium channel properties were investigated. All recordings were filtered at 1 kHz and stored at sampling rate of 10 kHz for offline analysis by PClamp 9. We also observed the purity of cell fraction by westernblotting . Protein samples preparations were subjected into SDS-PAGE and probed with specific antibodies: cox1, calnexin, 58kGolgi protein and actin.

Based on our previous published data, mitoBK channel is a non voltage-gated potassium channel at ±40 mV with a main conductance 565pS in 200 mM KCl cis/50 mM KCl trans which was blocked by IbTx (100 nM) and ATP (2.5mM). At present study, we found a similar channel which was inhibited by IbTx (100 nM) and ATP (2.5mM). Gating behavior showed that the channel open probability was voltage-dependent and the channel conductance decreased to 252 pS. Western blotting and antibodies directed against various cellular proteins revealed that mitochondrial inner membrane fractions did not contain specific proteins of the other subcellular compartments.

Our data showed that the biophysical properties of mitoBK channel were significantly altered in alzheimer's diease. Based on these findings, we propose that brain mito-BK channels are involved in cellular mechanisms of neurodegenera

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ANTI-AMYLOIDOGENIC POTITENTIAL OF NATURAL PRODUCTS: RELEVANCE TO ALZHEIMER'S DISEASE DRUG DISCOVERY

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Amyloid beta 42 (Abeta42) is the major etiological factor implicated in Alzheimer's disease (AD). Abeta(42) self-assembles to form oligomers and fibrils via multiple aggregation process. The recent studies aimed to decrease Abeta levels or prevention of Abeta aggregation which are the major targets for therapeutic intervention. Natural products as alternatives for AD drug discovery are a current trend. The studies on pharmacological properties of Caesalpinia crista (C. Crista) are very limited and this is the first report on the Anti-Alzheimer's potential of C. crista. Our study focused on ability of C. crista on the prevention of (i) the formation of oligomers and aggregates from monomers (Phase I: Abeta(42) + C.crista co-incubation); (ii) the formation of fibrils from oligomers (Phase II: C. crista added after oligomers formation);and (iii) dis-aggregation of pre-formed fibrils (Phase III: C. crista added to matured fibrils and incubated for 9 days). The aggregation kinetics was monitored using thioflavin-T assay and transmission electron microscopy (TEM). The results showed that C. crista could able to inhibit the Abeta(42) aggregation from monomers and oligomers and also able to dis-aggregate the pre-formed fibrils. The study provides an insight on finding novel natural products for AD drug discovery.

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P2X7 RECEPTOR ANTAGONISTS DELIVERED INTRAVITRALLY OR AS TOPICAL EYE-DROPS PROMOTE RETINAL GANGLION CELL SURVIVAL AND REGENERATION AFTER OPTIC NERVE INJURY

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Purpose: Neuronal degeneration after injury has been linked to activation of the P2X7 purinergic receptor. However, the role of P2X7 in retinal ganglion cell (RGC) death after optic nerve transection (ONT) remains unknown. We tested whether P2X7 blockade by specific antagonists could enhance RGC survival and axonal regeneration after ONT.

Methods: Adult hamsters were subjected to ONT 2mm behind the globe and injected intravitreally with P2X7 antagonists like Brilliant Blue Green (BBG), oxidized-ATP (Ox-ATP), A438079, A740003, or the vehicle solution 0.9% NaCl or DMSO. RGC survival was quantified by βIII tubulin immunostaining (TuJ1) at 7 days post-ONT. Two non-specific P2 antagonists, suramin and PPADS, were also assessed for their effects on RGC survival. The effects of BBG on RGC survival (at 7, 14 and 28 days post-ONT) were compared to intravitreal BDNF which is a potent neurotrophic factor for RGCs. In a separate study, BBG was applied as eye-drops twice a day after ONT to assess its effects on RGC survival. RGC axonal regeneration was studied by grafting a peripheral nerve (PN) to the cut ON together with intravitreal BBG or vehicle injection. The number of regenerating cells at 28 days post-grafting was determined by fluorescent dye retrograde labeling.

Results: Intravitreal BBG and A438079 promoted RGC survival dose-dependently, with a maximum of 93% at 7 days post-ONT, followed by Ox-ATP (89%) and A740003 (78%), whereas non-specific P2 antagonists like suramin (65%) and PPADS (61%) were only slightly better than the vehicle control (54%), suggesting that P2X7 blockade effectively promoted RGC survival. Except at 7 days post-ONT when the rescuing effect of BBG slightly fell short of BDNF (93% vs 99%), BBG promoted greater RGC survival than BDNF at 14 and 28 days. Interestingly, BBG given as eye-drops also enhanced survival from 7 to 28 days post-ONT, the magnitude of rescue being similar to intravitreal BBG. RGC axonal regeneration into PN graft was increased by 36% in BBG versus vehicle.

Conclusions: P2X7 blockade promotes long term RGC survival and increases axonal regeneration. BBG eye-drops also improve survival and may be useful as a non-invasive prolonged therapy for chronic diseases like glaucoma. (*Supported by GRF CUHK463309)*

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A CHINESE HERBAL DECOCTION KAI-XIN-SAN EXERTS ANTI-DEPRESSION EFFECT IN RATS EXPOSED TO CHRONIC MILD STRESS BY REGULATING NEUROTRANSMITTER AND NEUROTROPHIC FACTOR SYSTEMS IN THE BRAIN

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Kai-xin-san (KXS), a Chinese herbal formula firstly prescribed by Sun Simiao in Beiji Qianjin Yaofang about 1400 years ago, contains Ginseng Radix et Rhizome, Polygalae Radix, Acori Tatarinowii Rhizoma and Poria. KXS has been traditionally used to treat stress-related psychiatric disease with the symptoms of depression, forgetfulness and dizziness until nowadays. However, the mechanism of its anti-depression action is still unknown. Here, the chronic mild stress (CMS)-induced depressive rats were applied to explore the action mechanisms of KXS treatment. Daily intra-gastric administration of a chemically standardized KXS for four weeks significantly alleviated the CMS-induced depressive symptoms, which was displayed by the enhanced sucrose consumption of the rats. In addition, the expressions of various molecular bio-markers were alterted in the depressive rat brains by the treatment of KXS. These bio-markers included: (i) the levels of dopamine, norepinephrine and serotonin; (ii) the transcript levels of proteins relating to neurotransmitter metabolism; and (iii) the transcript levels of neurotrophic factors and their receptors. The results suggested that the anti-depressive action of KXS might be mediated by an increase of neurotransmitters and expression of neurotrophic factors and its corresponding receptors in the brain. Thus, this chemically standardized herbal extract could serve as alternative medicine or health food supplement for depressive

Acknowledgement: This research was supported by grants from Research Grants Council of Hong Kong (660409 and 660411 to RCYC; 662911 and S-HK005-11 to KWKT; 661110 to DTWL); Innovation and Technology Commission & Industry (GHP/030/09SZ to KWKT and GHP/032/09SZ to DTWL); and Chiu Chow Chamber of Commerce (Z0315 to KWKT).

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ACTIVATION OF UTP-SENSITIVE P2Y2 RECEPTOR INDUCES THE EXPRESSION OF CHOLINERGIC GENES IN CULTURED CORTICAL NEURONS: AN SIGNAL CASCADE TRIGGERED BY CA^{2+} MOBILIZATION AND ERK PHOSPHORYLATION

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Adenosine 5'-triphosphate (ATP), an extracellular signaling molecule, is co-stored and co-released with neurotransmitters at central and peripheral neuronal synapses, which ATP leads to the up-regulation of synaptic gene expression in muscles, including acetylcholine receptor and acetylcholinesterase (AChE) via P2Y receptor (P2YR). However, this trophic response was not determined yet in neurons. In cultured cortical neurons, different types of P2Y receptors were expressed which the UTP-sensitive $P2Y_2R$ was the most abundant. This result was consistent with that in rat brain during development. In cultured cortical neurons, $P2Y_2R$ was shown to be existed in membrane raft and co-localized with post-synaptic PSD-95 by immuno-cytofluorescent staining. Functionally, the activation of $P2Y_2R$ stimulated different cholinergic gene expressions including acetylcholinesterase (AChE), proline-rich membrane anchor (PRiMA) and choline acetyl-transferase (ChAT) in cortical neurons, which the signaling cascades involved Ca^{2+} mobilization and Erk1/2 activation. Lastly, the functional importance of post-synaptic $P2Y_2R$ was demonstrated by its synergistic effect with $P2Y_1R$ in controlling the expression of cholinergic genes. Taken together, the current results revealed a long term effect of $P2Y_2R$ in mediating synaptic gene expressions, and the co-activation effect between ATP and UTP in neuronal synapse.

Acknowledgement: This research was supported by grants from Research Grants Council of Hong Kong (660409 and 660411 to RCYC; 662911 and S-HK005-11 to KWKT) and Chiu Chow Chamber of Commerce (Z0315 to KWKT).

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BENEFICIAL EFFICACY OF FLAVONOIDS DERIVED FROM TRADITIONAL CHINESE MEDICINE: POTENTIAL DRUG DEVELOPMENT AGAINST DEPRESSION RELATED SYMPTOMS

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Flavonoids belong to a family of polyphenolic compounds. They are widely present in a variety of fruits, vegetables, tea, and wine, more importantly, many flavonoids serve as the active ingredients in traditional Chinese medicines (TCMs). Different lines of evidence indicated that flavonoids have impacts in many aspects of human health, including anti-tumor, anti-oxidation and anti-inflammation. Recently, numerous attentions focus on the neuro-beneficial effects of flavonoids including the neuroprotection against neurotoxin stress, promotion of memory, learning and cognitive functions. In this study, we focus on the nervous system, and aim to identify the effective flavonoids having the neuro-beneficial effects. Specifically, we target on induction of neurite outgrowth, modulation of synapse formation, and regulation of the synthesis and secretion of neurotrophic factors in astrocyte. After screening of 60 flavonoids, a flavonol aglycone called isorhamnetin was found to have a slight effect in promoting neurite outgrowth but promising effect in up-regulating neurofilament expressions in PC12 neuronal cells, the application of isorhamnetin could further potentiate the effect of nerve growth factor (NGF) in promoting neurite outgrowth. For synapse modulation, the flavonoids, catechin, luteolin, and isorhmanetin, were found to stimulate the expression of synaptic vesicle associated protein 48 (SV48), and post-synaptic density protein 95 (PSD-95) in cortical and hippocampal neurons. For neurotrophic factors regulation, we found that several flavonoids would be active in inducing the synthesis of neurotrophic factors. Based on all these findings, flavonoids possess multi-functional benefits to neurons and may provide a good and continuous resource for the new drug development against neurological disease.

Acknowledgement: This research was supported by grants from Research Grants Council of Hong Kong (660409 and 660411 to RCYC; 662911 and S-HK005-11 to KWKT; 661110 to DTWL); Innovation and Technology Commission & Industry (GHP/030/09SZ to KWKT and GHP/032/09SZ to DTWL); and Chiu Chow Chamber of Commerce (Z0315 to KWKT).

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MOLECULAR ASSEMBLY OF PRIMA-LINKED ACETYLCHOLINESTERASE TETRAMER: THE N-GLYCOSYLATION ON PRIMA AND CATALYTIC SUBUNITS DURING BIOSYNTHESIS

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In the brain, acetylcholinesterase (AChE) anchors onto cell membranes by a transmembrane protein PRiMA (proline-rich membrane anchor), as a tetrameric (G_4) form. The assembly of G_4 AChE with PRiMA requires a C-terminal "t-peptide" in the AChE catalytic subunit ($AChE_T$). Although AChE is a well-known N-glycoprotein, the role of glycosylation in forming the physiologically active PRiMA-linked G_4 AChE remains unclear. Here, the glycosylation of $AChE_T$ and PRiMA subunits were determined in directing the oligomerization, enzymatic activity and membrane trafficking of G_4 AChE complex. Using the N-glycosylation site mutants of $AChE_T$ and PRiMA, we showed that human $AChE_T$ contained three N-glycosylation sites located at 296, 381 and 495 residues, and mouse PRiMA possessed a single N-glycosylation site located at 43 residue. By co-expressing $AChE_T$ glycosylation mutants and wild type PRiMA in HEK293T cells, we found that the N-linked glycosylation of $AChE_T$ played a major role for acquisition of full enzymatic activity of G_4 AChE, but which did not affect its oligomerization with PRiMA. The glycan-depleted G_4 AChE was assembled in endoplasmic reticulum, but which was not transported to Golgi apparatus or plasma membrane. On other hand, the N-linked glycosylation of PRiMA, when co-expressing wild type $AChE_T$, was not essential for the oligomerization, enzymatic activity and protein trafficking of G_4 AChE complex. The importance of N-glycosylation of $AChE_T$ subunits in G_4

AChE complex strongly suggests that the regulating elements (possibly chaperons in ER) could recognize glycans on $AChE_T$ subunits, and these elements assist its membrane trafficking. Thus, a functional G_4 AChE could be localized on the plasma membrane.
Acknowledgement: This research was supported by grants from Research Grants Council of Hong Kong (660409 and 660411 to RCYC; 662911 and S-HK005-11 to KWKT) and Chiu Chow Chamber of Commerce (Z0315 to KWKT).

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THE ROLE OF GLYCOSYLATION AND CARBOXYLIC TERMINUS IN THE MOLECULAR ASSEMBLY OF DIMERIC ACETYLCHOLINESTERASE IN ERYTHROCYTE

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Acetylcholinesterase (AChE) is crucial in the nervous system for its cholinergic functions. However, AChE is also present in other tissues where its function remains unknown. Through alternative splicing, AChE is synthesized as different splicing variants with different isoforms, including tailed (T), read-through (R) and hydrophobic (H) forms. AChEH form exists as a GPI-linked dimer on the plasma membrane of human erythrocyte. Here, we aimed to reveal the assembly and trafficking of AChEH dimer. In cultured HEK293T cells, the recombinant AChEH was functionally expressed in dimeric form and glycosylated. The same result was confirmed in erythroid precursor cell line TF-1 and erythrocytes. The function of glycosylation was studied by mutating all three putative glycosylation sites on the enzyme. The triple glycosylation mutant of AChEH showed complete removal of glycans, which was supported by glycosidases treatment. To reveal the functions of glycosylation, enzyme actvity assays performed showed that the glycan-deprived AChEH mutant had lower catalytic activty than that of the wild type enzyme. Interestingly, the AChE glycosylation mutant could still be assembled into dimer in the cells, but retained mostly in endoplasmic reticulum (ER) and could not be trafficked to Golgi apparatus and plasma membrane. These results suggested that glycosylation of AChEH affected the catalytic activity of the enzyme as well as the protein trafficking, particular during the assembly process from ER to plasma membrane; however, the formation of dimer was not affected. The present study indicated the significance of post-translational modification in controlling the biosynthesis of AChEH.
Acknowledgement: This research was supported by grants from Research Grants Council of Hong Kong (660409 and 660411 to RCYC; 662911 and S-HK005-11 to KWKT) and Chiu Chow Chamber of Commerce (Z0315 to KWKT).

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A NOVEL CARBAZOLE-BASED FLUOROPHORE INHIBIT AGGREGATIONS OF BETA-AMYLOID PEPTIDE

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Aggregation of monomeric Aβ peptides to insoluble plaque-associated amyloid fibrils via soluble oligomeric intermediates would induce a cascade of events that eventually lead to neuronal cells death. This is one of the key cascades in Alzheimer's disease (AD) pathogenesis. As a results, interferes with the aggregation of the Aβ peptides, which further inhibiting the formation of the neurotoxic oligomers and fibrils, is an attractive primary approach to the treatment of AD. In the present study, multifunctional carbazole-based fluorophores, SLOH, was synthesis and was applied on cells and transgenic animal models. The cytotoxicity of SLOH towards human neuroblastoma SH-SY5Y neuronal cells exposed for 2, 6 and 24 h were measured by using the MTT assay. No significant cytotoxicity (< 20 %) at concentrations of ≤ 1 μM from 2 to 24 h of exposure was found. The effect of SLOH on three different forms of Aβ(1–40) peptide (Aβ(1–40) peptide monomer (M), Aβ(1–40) peptide monomer with seeded fibril (MS), and Aβ(1–40) fibril (F))-induced cytotoxicity on the SH-SY5Y cells and mouse primary cortical cells on three different time points (2, 6 and 24 h), in three different [SLOH]/[Aβ] ratio (0.2, 1.0 and 5) were examined.

The results showed a neuroprotective effect of SLOH in the cytotoxic activities induced by all three forms of Aβ(1–40) peptide and at [SLOH]/[Aβ] of 0.2, 1.0 and 5 for both SH-SY5Y cells and primary cells after 2 h of incubation. Moreover, tail vein injection of SLOH was performed. The fluorescence images of the tissue co-stained with the Aβ labeling dye, ThT or the immunohistochemistry images of the tissue co-stained with Aβ antibody indicated that SLOH was able to pass through the BBB. These results suggested that SLOH was able to targets the Aβ plaque and can be a potential neuroprotective and therapeutic agent for Alzheimer's disease.

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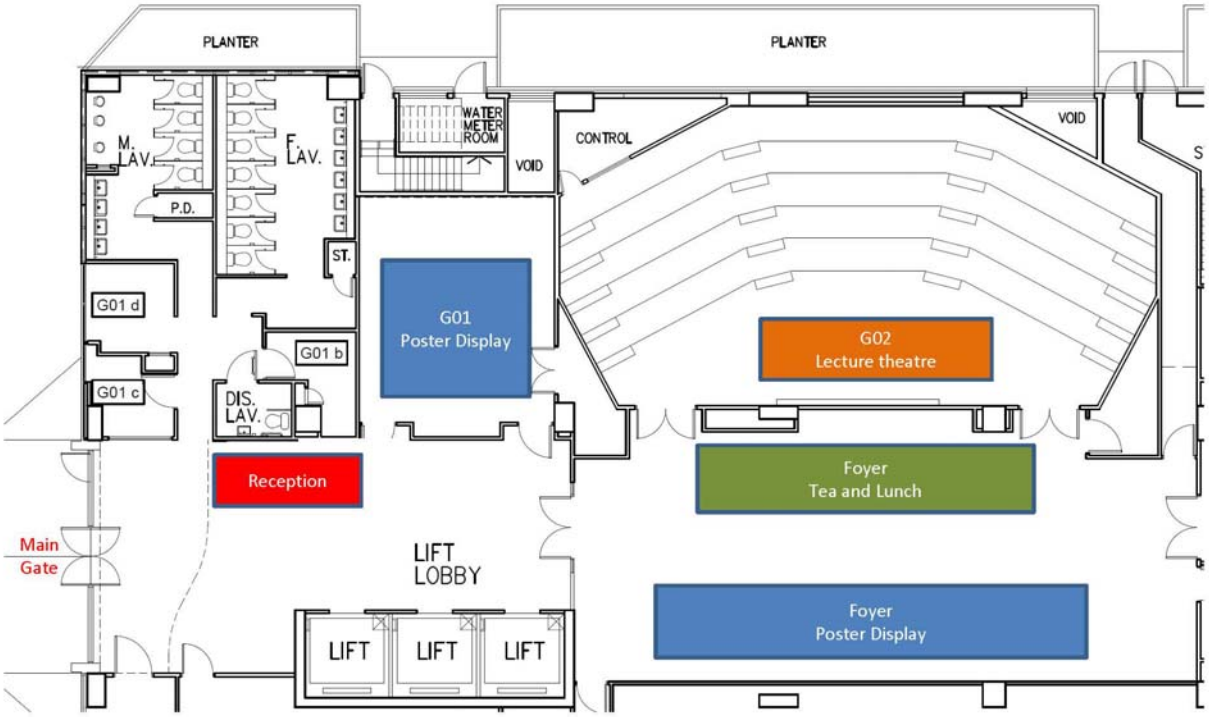

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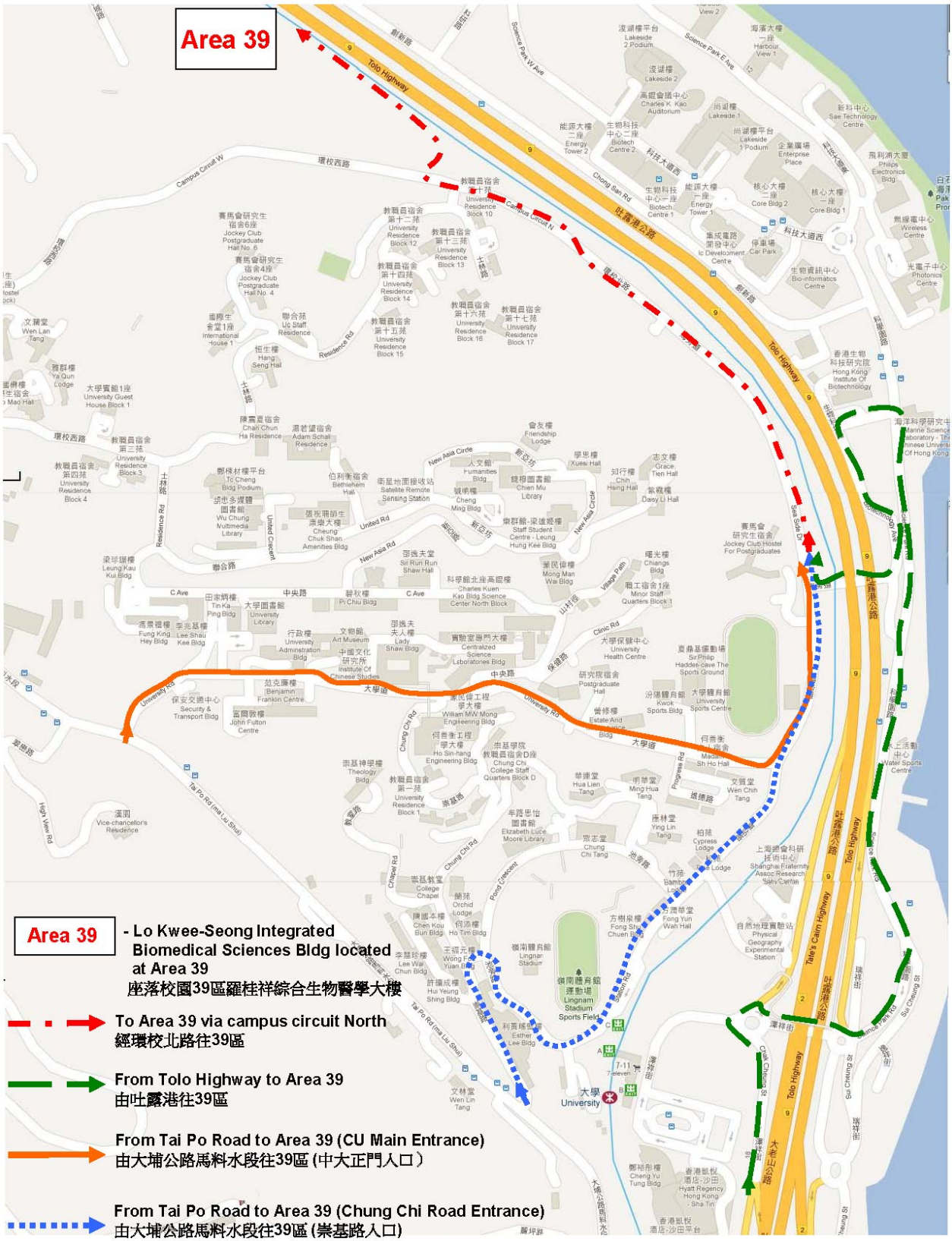

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A coach especially arranged by the organizer will take participants from the University Train Station to the LKS Building **between 7:45 am and 8:45 am** on June 14th and June 15th, and **between 1:00 pm and 1:45 pm** on June 14th

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