School of Biomedical Sciences Research Day 2012

&

Cancer and Inflammation 2012 Symposium

4-5, June, 2012

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

香港中文大學
The Chinese University of Hong Kong

FACULTY OF MEDICINE
THE CHINESE UNIVERSITY OF HONG KONG

School of Biomedical Sciences Research Day 2012

Members of the Organizing Committee

Professor Franky L. Chan

Professor Wai Yee Chan

Professor Chi Hin Cho

Professor Yiu Wa Kwan

Professor Alaster H.Y. Lau

Professor Kingston K.L. Mak

Professor Chao Wan

Professor Hui Zhao

Welcome Message from the Director of School of Biomedical Sciences

The School of Biomedical Sciences has undergone a major transformation this year. When the School was formed in 2009, our staff was scattered in different buildings. Now, for the first time in the history of the Faculty of Medicine at CUHK, all pre-clinical staff are together under one roof. This is a true milestone in the development of the School. I would like to extend the warmest welcome to all of our Associate Members and guests, and encourage you to take a walk through this new home of ours while you are attending this Research Day.

This Research Day also marks new beginnings on a number of fronts. Aside from being the first Research Day held in our own building, we are trying out a new program format for Research Day this year. Unlike previous Research Days, members of two of the five Thematic Programs will present their work on the podium, while the other three Thematic Programs will present their work using posters. Next year, the three Thematic Programs that present posters this year will deliver oral presentation, while the two Programs presenting orally this year will present posters. We hope this format will give more time for the oral presentations and thus, increase the interaction between the presenter and the audience. This year, in addition to our School Research Day, we will be holding a joint symposium with the Cancer Institute subsequent to the Research Day. It is our hope that the Research Day will serve as a platform for interaction between our members and scientists outside in addition to its role as a platform for exchange of ideas for our School members. We hope you like this new format. I am sure our Organizing Committee will appreciate any input you care to give us.

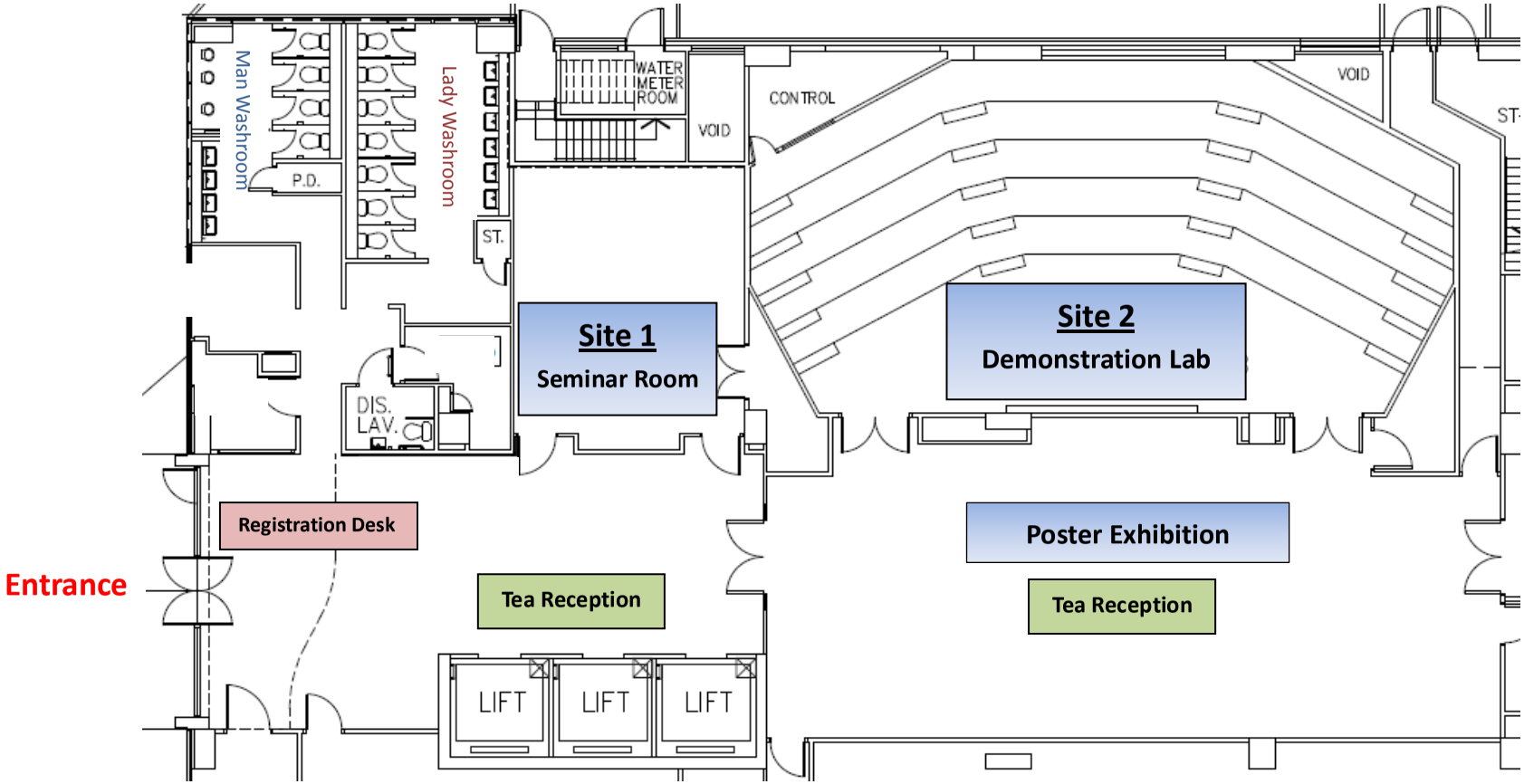
With the exception of the Chair, the Organizing Committee was made up of all fresh members this year. This injection of new blood is very important for the continued success of the Research Day. I would like to thank Professor C.H. Cho and all members of the Organizing Committee for their hard-work. In particular, I would like to thank Joresa and Isabel for giving such great support to the Organizing Committee.

With a new building and all members of the School together, we are set to take the journey to success that starts today. It is my hope that we will be able to claim proudly in the not so distant future that our School is one of the centers of great science in the region and in the world.

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

Map of the Meeting Venue

G/F Lo Kwee-Seong Integrated Biomedical Sciences Building



Programme Summary

SBS Research Day 2012

4 June, 2012 (Monday)

09:00-09:15 Opening Ceremony (Dean and Director)
09:15-09:30 Photo taking

09:30-10:15 Plenary Lecture by Prof. Ye Guang Chen, Demonstration Lab (Rm G02)

Time	*Seminar Room (Rm G01)*	*Demonstration Lab (Rm G02)*
10:15-11:05	**Cancer and Inflammation (1): Inflammation and cancer therapy** (Franky L. Chan and Paul B.S. Lai)	**Reproduction, Development, and Endocrinology (1): Stem cell and differentiation** (Christopher H.K. Cheng and Pak Cheung Ng)
10:15-10:40	Prof. Kwok Pui Fung / Dr. Judy Chan	Prof. Pak Cheung Ng
10:40-11:05	Prof. Alaster H.Y. Lau	Prof. Tin Lap Lee

11:05-11:45 ***Tea Break & Poster Viewing***

11:45-13:00	**Cancer and Inflammation (2): Inflammation related signaling and anti-cancer targets** (Ken W.K. Liu and Qian Tao)	**Reproduction, Development, and Endocrinology (2): Lineage differentiation and cell signaling in development** (Wing Hung Ko and Juliana C.N. Chan)
11:45-12:10	Prof. Michael S.C. Tam	Prof. Po Sing Leung
12:10-12:35	Prof. Ge Lin	Prof. Woody W.Y. Chan
12:35-13:00	Prof. Gang Li	Prof. Yin Xia

13:00-14:00 ***Lunch Break***
14:00-15:00 ***Poster Presentation Session***

15:00-16:15	**Cancer and Inflammation (3): Anti-cancer targets/therapy** (Stephen K.W. Tsui and Kwok Wai Lo)	**Reproduction, Development, and Endocrinology (3): Cell signaling and gene targeting in development** (Po Sing Leung and Richard K.W. Choy)
15:00-15:25	Prof. Kenneth K.W. To	Prof. Sidney S.B. Yu
15:25-15:50	Prof. Ken W.K. Liu	Prof. Hui Zhao
15:50-16:15	Prof. Tzi Bun Ng	Prof. Christopher H.K. Cheng

16:15-16:45 ***Tea Break & Poster Viewing***

16:45-17:35	**Cancer and Inflammation (4): Tumor suppressor and gene delivery** (Tzi Bun Ng and Nathalie Wong)	**Reproduction, Development, and Endocrinology (4): Disease and embryopathy** (Woody W.Y. Chan and Ronald C.W. Ma)
16:45-17:10	Prof. Hsiang Fu Kung / Dr. Hong Yao	Prof. Wing Hung Ko
17:10-17:35	Prof. Hsiao Chang Chan	Prof. Alisa S.W. Shum

17:45-18:45 **SBS Tour - Assemble outside Demonstration Lab, G/F**
19:00 **Conference Dinner (by invitation)**

Site 1 Seminar Room (Room G01), Lo Kwee-Seong Integrated Biomedical Sciences Building

09:00-09:15 Opening Ceremony: Prof. Tai Fai Fok (Dean of Medicine) & Prof. Wai Yee Chan (Director of School of Biomedical Sciences), Demonstration Lab (Rm G02), Lo Kwee-Seong Integrated Biomedical Sciences Building

Time	*Title of Presentation*	*Name of Speaker*	*Abstract No.*
10:15-11:05	**Cancer and Inflammation (1): Inflammation and cancer therapy** **Chairpersons: Prof. Franky L. Chan and Prof. Paul B.S. Lai**		
10:15-10:40	Mechanistic studies of photodynamic therapy of pheophorbide a on human breast tumour in *in vitro* and *in vivo* models	Prof. Kwok Pui Fung / Dr. Judy Chan	S1-01
10:40-11:05	Pharmacology of adenosine receptors in human mast cells	Prof. Alaster H.Y. Lau	S1-02
11:05-11:45	***Tea Break & Poster Viewing***		
11:45-13:00	**Cancer and Inflammation (2): Inflammation related signaling and anti-cancer targets** **Chairpersons: Prof. Ken W.K. Liu and Prof. Qian Tao**		
11:45-12:10	The role of NF-κB in the anti-HSV-1 activity of trichosanthin	Prof. Michael S.C. Tam	S1-03
12:10-12:35	Potential biomarker for the assessment of pyrrolizidine alkaloids-induced hepatotoxicity	Prof. Ge Lin	S1-04
12:35-13:00	Inhibiting CD164 expression in colon cancer cell line HCT116 leads to reduced cancer cell proliferation, mobility and metastasis *in vitro* and *in vivo*	Prof. Gang Li	S1-05
13:00-14:00	***Lunch Break***		
14:00-15:00	***Poster Presentation Session***		
15:00-16:15	**Cancer and Inflammation (3): Anti-cancer targets/therapy** **Chairpersons: Prof. Stephen K.W. Tsui and Prof. Kwok Wai Lo**		
15:00-15:25	Multidrug resistant (MDR) cancer cell-targeting effect by PPARγ partial agonists	Prof. Kenneth K.W. To	S1-06
15:25-15:50	Glycosylation and melanoma	Prof. Ken W.K. Liu	S1-07
15:50-16:15	A type II ribosome inactivating protein and a ribonuclease with antitumor activity from the bitter gourd	Prof. Tzi Bun Ng	S1-08
16:15-16:45	***Tea Break & Poster Viewing***		
16:45-17:35	**Cancer and Inflammation (4): Tumor suppressor and gene delivery** **Chairpersons: Prof. Tzi Bun Ng and Prof. Nathalie Wong**		
16:45-17:10	PEI-CyD-FA coated adenovirus as an effective and safe gene delivery vector	Prof. Hsiang Fu Kung / Dr. Hong Yao	S1-09
17:10-17:35	CFTR as a tumor suppressor and prognosis indicator	Prof. Hsiao Chang Chan	S1-10

17:45-18:45 **SBS Tour - Assemble outside Demonstration Lab (Rm G02), G/F**

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

Site 2 Demonstration Lab (Room G02), Lo Kwee-Seong Integrated Biomedical Sciences Building

09:00-09:15 Opening Ceremony: Prof. Tai Fai Fok (Dean of Medicine) & Prof. Wai Yee Chan (Director of School of Biomedical Sciences), Demonstration Lab (Rm G02), Lo Kwee-Seong Integrated Biomedical Sciences Building

09:30-10:15 Plenary Lecture by Prof. Ye Guang Chen (Abstract no. PL-01)
"Modulation of mouse embryonic stem cell fate by BMP"

Time	*Title of Presentation*	*Name of Speaker*	*Abstract No.*
10:15-11:05	**Reproduction, Development, and Endocrinology (1): Stem cell and differentiation Chairpersons: Prof. Christopher H.K. Cheng and Prof. Pak Cheung Ng**		
10:15-10:40	Biomarkers of Neonatal Infection and NEC – The State of the Art Approach	Prof. Pak Cheung Ng	S2-01
10:40-11:05	Age-dependent regulation of spermatogonial stem development by novel long non-coding RNAs	Prof. Tin Lap Lee	S2-02

11:05-11:45 ***Tea Break & Poster Viewing***

11:45-13:00	**Reproduction, Development, and Endocrinology (2): Lineage differentiation and cell signaling in development Chairpersons: Prof. Wing Hung Ko and Prof. Juliana C.N. Chan**		
11:45-12:10	Angiotensin II-AT2 receptor axis is involved in the functional maturation of human pancreatic progenitor cells toward the endocrine lineage	Prof. Po Sing Leung	S2-03
12:10-12:35	Dab2 is a regulator of skeletal muscle development	Prof. Woody W.Y. Chan	S2-04
12:35-13:00	Deletion of Dragon reveals the different roles of IL-6, TNF-α and IL-1β in inhibiting growth hormone signaling in the liver	Prof. Yin Xia	S2-05

13:00-14:00 ***Lunch Break***

14:00-15:00 ***Poster Presentation Session***

15:00-16:15	**Reproduction, Development, and Endocrinology (3): Cell signaling and gene targeting in development Chairpersons: Prof. Po Sing Leung and Prof. Richard K.W. Choy**		
15:00-15:25	Interaction between TRAPP and dynactin demonstrates a novel function of TRAPP in mammalian COPII vesicle tethering	Prof. Sidney S.B. Yu	S2-06
15:25-15:50	Transcription factor Ets-1 plays dual roles in neural crest formation	Prof. Hui Zhao	S2-07
15:50-16:15	A robust platform for efficient targeted gene disruption in zebrafish using TALENs	Prof. Christopher H.K. Cheng	S2-08

16:15-16:45 ***Tea Break & Poster Viewing***

16:45-17:35	**Reproduction, Development, and Endocrinology (4): Disease and embryopathy Chairpersons: Prof. Woody W.Y. Chan and Prof. Ronald C.W. Ma**		
16:45-17:10	P2Y receptors in human airway epithelia: from ion transport to airway inflammation	Prof. Wing Hung Ko	S2-09
17:10-17:35	Too much causes too little - a new teratogenic mechanism by retinoic acid	Prof. Alisa S.W. Shum	S2-10

17:45-18:45 **SBS Tour – Assemble outside Demonstration Lab (Rm G02), G/F**
19:00 **Conference Dinner (by invitation)**

Oral Presentations

Abstracts

Modulation of mouse embryonic stem cell fate by BMP

Y.G. Chen, F. Teng, Z. Li

The State Key Laboratory of Biomembrane and Membrane Biotechnology, School of Life Sciences, Tsinghua University, Beijing 100084, China.

Embryonic stem (ES) cells are under precise control of both intrinsic self-renewal gene regulatory network and extrinsic growth factor-triggered signaling cascades. How external signaling pathways connect to intracellular regulatory circuits is largely unknown. To probe this, we choose BMP signaling which is previously recognized as a master control for both self-renewal and lineage commitment of mouse ES cells. Mapping of target gene promoter occupancy of Smad1/5 and Smad4 on a genome-wide scale revealed that these Smad proteins associate with a large group of developmental regulators. Smad-mediated BMP signaling is largely required for differentiation-related processes rather than directly influences self-renewal. Our results suggest that Smad-mediated BMP signaling balances self-renewal versus differentiation by modulating a set of developmental regulators. In addition, we found that while LIF signaling augments ERK activity, BMP signaling inhibits ERK activity in mouse ES cells via direct upregulation of an ERK phosphatase – DUSP9. The cooperative effects of LIF and BMP signaling keep appropriate ERK activity and maintain mouse ES cell self-renewal. These findings shed light on how extrinsic signals converge to intrinsic signaling molecules to regulate cell fate determination.

Mechanistic studies of photodynamic therapy of pheophorbide a on human breast tumour in *in vitro* and *in vivo* models

N.H. Bui-Xuan[1], J.Y.W. Chan[2,3], S.W.H. Hoi[2,3], J.L. Jiang[2,3], H.M. Wong[1], K.K.Y. Cheung[1], C.K. Wong[2,3,4], K.P. Fung[1,2,3]

[1]School of Biomedical Sciences, [2]Institute of Chinese Medicine, [3]State Key Laboratory of Phytochemistry and Plant Resources in West China (CUHK) , [4]Department of Chemical Pathology, The Chinese University of Hong Kong (CUHK), Hong Kong SAR, China.

Breast cancer is conventionally treated by surgery and radiotherapy, supported by adjuvant chemotherapy or hormonotherapy. However, adverse side effects and drug resistance may be resulted in patients. We examined the possibility of using photodynamic therapy (PDT), which requires a photosensitizer, upon the activation of visible light, to treat breast cancer. In this study, the anti-proliferative effect and underlying mechanisms of Pheophorbide a (Pa), a photosensitizer isolated from Traditional Chinese Medicine *Scutelleria barbata*, on human breast tumour cells were investigated. The IC_{50} value on human breast tumour MCF-7 cells was found to be 0.5 μM when incubated with the cells for 24 hours after photodynamic therapy with Pa (Pa-PDT). Mechanistic studies demonstrated that Pa was localized in the mitochondria and reactive oxygen species were found to be released after Pa-PDT on MCF-7 cells. As evidenced by the results of cell death detection ELISA and chromatin condensation detected by Hoechst 33342 staining, apoptosis was found to be the major mechanism for the tumour cell death. Mitochondrial membrane depolarization and cytochrome *c* release revealed the role of mitochondria in the apoptotic mechanism. Increased expression of tumour suppressor protein p53, cleavage of caspase-9, caspase-7 and Poly (ADP-ribose) polymerase indicated the involvement of caspase-dependent pathway. On the other hand, apoptosis-inducing factor release demonstrated the mediation of caspase-independent mechanism. *In vivo* study using the mouse xenograft model showed a significant inhibition of tumour growth by Pa-PDT in nude mice bearing MCF-7 cells. Pa-PDT could also exhibit anti-angiogenesis activities. The probable immunomodulatory effect of Pa, in the absence of irradiation by visible light, was further investigated in mouse macrophage RAW264.7 cells. Pa could significantly induce the release of interleukine-6 and tumour necrosis factor-α, and enhance the phagocytic activities of RAW264.7 cells. Our results in present study provide a basis for further developing Pa-PDT as one of modalities to treat breast cancer.

Pharmacology of adenosine receptors in human mast cells

K.H. Yip, H. Wise, H.Y.A. Lau

School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong.

Interaction of adenosine and mast cells contribute to the pathophysiology of inflammatory reactions such as asthma. We employed peripheral blood $CD34^+$ monocytes derived human cultured mast cells (HCMC) to elucidate the pharmacology of adenosine receptors involved. RT-PCR analysis and immunofluorescence staining studies respectively identified the expression of mRNAs and proteins for all four subtypes of adenosine receptor (A_1, A_{2A}, A_{2B} and A_3 ARs) in HCMC. Functional studies employing agonists and antagonists of various adenosine receptors demonstrated that when HCMC were activated by anti-IgE after 10 min preincubation with adenosine, a biphasic response on histamine and IL-8 release was observed with enhancement of HCMC activation at low concentrations of adenosine (10^{-9}–10^{-7} M) mediated through A_1 AR and inhibition at higher concentrations (10^{-6}–10^{-4} M) through A_{2B} AR. Study on intracellular calcium concentration ($[Ca^{2+}]_i$) revealed that 10^{-8} M of adenosine through activation of PI3Kγ significantly enhanced anti-IgE induced Ca^{2+} influx. In contrast, adenosine at 10^{-4} M substantially inhibited $[Ca^{2+}]_i$ in response to anti-IgE. Furthermore, investigation on intracellular signalling molecules provided evidence that adenosine at concentrations over 10^{-6} M inhibited the anti-IgE activation of ERK, JNK or NF-κB pathways, whereas enhancement of IκBα was found with low concentration of adenosine. The above observations further justified the dual action of adenosine on anti-IgE-induced mediators release from HCMC.

This work was fully supported by the Research Grants Council of Hong Kong (CUHK4515/06M)

The role of NF-κB in the anti-HSV-1 activity of trichosanthin

M.S.C. Tam, D. He

School of Biomedical Sciences, The Chinese University of Hong Kong.

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

Potential biomarker for the assessment of pyrrolizidine alkaloids-induced hepatotoxicity

G. Lin, N. Li, J.Q. Ruan

School of Biomedical Sciences, The Chinese University of Hong Kong.

With the rapidly growing global interests in the use of natural products including medicinal herbs, their safety issue attracts more public concerns. Among various phytotoxins, pyrrolizidine alkaloids (PA), which naturally and diversely distribute across the plant kingdom, are one of the most common causes of food and/or herb poisonings. Most of the naturally occurring PAs are hepatotoxic, causing hepatic sinusoidal obstruction syndrome (HSOS), and may also induce liver cancer after prolonged exposure. Regardless of structural differences, all hepatotoxic PAs undergo P450-mediated metabolic activation to generate corresponding pyrrole metabolites, which further react with proteins or DNAs to form pyrrole-protein or pyrrole-DNA adducts, leading to toxicity to cancer in the liver. To date, there is no available method to specifically diagnose and assess the toxicity induced by PAs. In this presentation, the investigation of the mechanism of PA-induced hepatotoxicity and the development of a mechanism-based biomarker for the assessment of such toxicity will be addressed. In addition, a successful application of our established method with the developed biomarker for definitive diagnosis and assessment of hepatotoxicity in HSOS patents poisoned by PA-containing herbs or PA-contaminated food stuffs will be discussed. [Supported by Research Grant Council of Hong Kong (GRF Grant CUHK471310)]

Inhibiting CD164 expression in colon cancer cell line HCT116 leads to reduced cancer cell proliferation, mobility and metastasis *in vitro* and *in vivo*

J. Tang[1], G. Zhou[2], J. Xiang[1], G. Li[3]

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[2] School of Medicine, Shenzhen University, Shenzhen, PR China.
[3] Stem Cell and Regeneration Program, School of Biomedical Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong.

Background: CD164 (Endolyn) is a sialomucin which has been found to play roles in regulating proliferation, adhesion and differentiation of hematopoietic stem cells. Possible association of CD164 with solid cancer development remains unknown.

Methods and Results: We first studied CD164 expression in biopsies from colorectal cancer, breast and ovary cancer patients by semi-quantitative immunohistochemistry, and found that CD164 was strongly expressed in all the colorectal cancer samples compared to the matching normal colon tissues. The possible roles of CD164 in colon cancer development were further investigated using a well-established human colon cancer cell line HCT116. We found that knockdown of CD164 expression in HCT116 cells significantly inhibited cell proliferation, mobility and metastasis in vitro and in vivo. The knockdown of CD164 expression was associated with decreased chemokine receptor CXCR4 expression HCT116 cell surface and immunoprecipitation studies showed that CD164 formed complexes with CXCR4.

Conclusions: CD164 is highly expressed in the colon cancer sites, and it promotes HCT116 colon cancer cell proliferation and metastasis both in vitro and in vivo, and the effects may act through regulating CXCR4 signaling pathway. Therefore, CD164 may be a new target for diagnosis and treatment for colon cancer.

Multidrug resistant (MDR) cancer cell-targeting effect by PPARγ partial agonists

K.K. To[1], B. Tomlinson[2]

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Multidrug resistance (MDR) remains an unresolved clinical problem hindering successful chemotherapeutic treatment of cancers. It is often associated with increased efflux of a variety of structurally unrelated anticancer drugs by ATP-binding cassette (ABC) transporters such as P-gp, ABCG2 and MRP1. There has been great interest in the development of inhibitors towards these transporters in order to stop the drug efflux and reverse drug resistance. However, since the inhibition of transporter is not specific to cancer cells, it could also lead to impairment of the natural protective mechanism in the gastrointestinal tract and the drug excretion process in the liver. As a result, a decrease in the cytotoxic drug dosing may be needed to prevent excess toxicity, thus undermining the potential benefit obtained from having a drug efflux inhibitor. The design of potent MDR modulators specific towards the resistant cancer cells and devoid of drug-drug interactions will be highly desirable to effect reversal of MDR.

Telmisartan is a well-tolerated angiotensin II receptor blocking anti-hypertensive agent in clinical use. Previous studies have indicated that it has moderate inhibitory activity on ABCG2-mediated drug efflux. Prompted by the fact that telmisartan is generally considered devoid of drug-drug interaction, we sought to investigate if it can be used to circumvent MDR without the aforementioned problem. We have observed the remarkable PTEN loss in ABCG2-overexpressing resistant cancer cell lines, which mediate the preferential expression of ABCG2 on cell membrane via the PI3K—Akt pathway. Interestingly, telmisartan is a peroxisome proliferators-activated receptor (PPAR . partial agonist, which was found to upregulate PTEN transcriptionally in the resistant cells, thereby leading to the migration of ABCG2 expression back to the cytoplasm and an apparent circumvention of ABCG2-mediated MDR. Since this novel ABCG2 regulatory pathway is only functional in drug-resistant cancer cells with PTEN loss, telmisartan may represent a promising resistant cell-targeting agent for MDR reversal.

Glycosylation and Melanoma

W.K. Liu, F.W.K. Cheung, Y.H. Ling

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Melanoma is one of the malignant cancers in Caucasian populations that causes about 65% of the mortality by skin cancers, but effective treatment is limited by its rapid metastasis, low response rates and fast development of resistance to chemotherapy. New strategies and more potent chemotherapeutic agents are urged to attenuate this highly fetal disease. About 50% of proteins in melanoma cells are synthesized and glycosylated in a form of post-translational modification in the rough endoplasmic reticulum (ER) before they are secreted to the Golgi and target organelles. Glycosylation is an enzymatic process through which an oligosaccharide is conjugated to a protein for different physiological and pathological events. Aberrant glycosylation not only interferes with protein maturation but also initiates ER stress and unfolded protein responses, and triggers the consequent cell death. Interruption of glycosylation has been a novel therapeutic strategy for melanoma. In a continuing search for bioactive natural products, it was found that isomalabaricanes, a small class of rearranged triterpene metabolites obtained from marine sponges, inhibited the growth of melanoma cells by an induction of abnormal protein glycosylation, ER stress and cell death.

A type II ribosome inactivating protein and a ribonuclease with antitumor activity from the bitter gourd

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

School of Biomedical Sciences, The Chinese University of Hong Kong.

Ribonucleases (RNases) are ubiquitously distributed nucleases that cleave RNA into smaller pieces. They are promising drugs for different cancers based on their concrete antitumor activities in vitro and in vivo. A14-kDa RNase, designated as RNase MC2, in the seeds of bitter gourd (Momordica charantia) manifested both cytostatic and cytotoxic activities against MCF-7 breast cancer cells. Treatment of MCF-7 cells with RNase MC2 caused nuclear damage (karyorrhexis, chromatin condensation, and DNA fragmentation), ultimately resulting in early/late apoptosis. Further molecular studies unveiled that RNase MC2 induced differential activation of MAPKs (p38, JNK and ERK) and Akt. On the other hand, RNase MC2 exposure activated caspase-8, caspase-9, caspase-7, increased the production of Bak and cleaved PARP, which in turn contributed to the apoptotic response.

In this study, the antitumor activity of Momordica charantia lectin (MCL), a type II ribosome inactivating protein from bitter gourd, on nasopharyngeal carcinoma (NPC) was also investigated. MCL evinced potent cytotoxicity toward NPC CNE-1 (IC(50) = 6.9) and CNE-2 (IC(50) = 7.4) cells but minimally affected normal NP 69 cells. Further investigation disclosed that MCL induced apoptosis, DNA fragmentation, G(1)-phase arrest, and mitochondrial injury in both types of NPC cells. The reduction of cyclin D1 and phosphoretinoblastoma (Rb) protein expression contributed to arrest at G(1)-phase of the cell cycle. These events were associated with regulation of mitogen-activated protein kinases (MAPK; including p38 MAPK, JNK, and ERK) phosphorylation and promoted downstream nitric oxide (NO) production. Concurrent administration of the p38 MAPK inhibitor SB-203580 significantly diminished NO production and lethality of MCL toward NPC cells. Further studies revealed that MCL increased cytochrome c release into the cytosol, activated caspases-8, -9, and -3, and enhanced production of cleaved PARP, subsequently leading to DNA fragmentation and apoptosis. Finally, an intraperitoneal injection of MCL (1.0 mg/kg/d) led to an average of 45% remission of NPC xenograft tumors subcutaneously inoculated in nude mice.

PEI-CyD-FA coated adenovirus as an effective and safe gene delivery vector

H. Yao, Marie C. Lin, H.F. Kung

School of Biomedical Sciences, The Chinese University of Hong Kong.

Recombinant adenovirus is evolving as a promising gene delivery vector for gene therapy. However, the host immune response could cause rapid clearance of the vector, impair its efficacy, and obstruct its effective clinical applications. We have previously synthesized a biodegradable co-polymer consisting of low molecular weight PEI, β-cyclodextrin, and folic acid (PEI-CyD-FA). Here we reported that adenovirus coated with PEI-CyD-FA could enhance the transfection efficiency and prolonged the duration of gene expression in immuno-competent mice by either intratumoral injection and systemic administration. Importantly, repeated injection did not reduce the transfection efficiency. Furthermore, we found that PEI-CyD-FA may coat the capsid protein of adenovirus and transformed the surface charge of adenovirus capsomers from negative to positive in physiological solution, which could shelter the epitopes of capsid protein of adenovirus. These findings suggest that PEI-CyD-FA coated Adv is a promising gene delivery system and warrant further investigations.

CFTR as a tumor suppressor and prognosis indicator

H.C. Chan, X.H. Jiang

Epithelial Cell Biology Research Center, School of Biomedical Sciences, Faculty of Medicine The Chinese University of Hong Kong.

Accumulating reports have indicated the association of cancer incidence with genetic variations in CFTR gene. However, the exact role of CFTR in cancer development, and the possible underlying mechanism have not been elucidated. Our recent studies have shown that CFTR expression is significantly decreased in human prostate, breast and colon cancers. Over-expression of CFTR in the cancer cells suppresses tumor progression (cell growth, adhesion and migration), whereas knockdown of CFTR leads to enhanced malignancies both *in vitro* and *in vivo*. In addition, we demonstrated that CFTR knockdown-enhanced cell invasion and migration are significantly reversed by antibodies against either uPA or uPAR, which are known to be involved in various malignant traits of cancer development. More interestingly, CFTR appears to regulate cancer malignancies through a number of different mechanisms, including protein-protein interaction with junctional complex proteins, negative regulation of NF-kB-COX2 pathway and alteration of microRNAs. We also found that downregulation of CFTR is associated with poor prognosis of cancers in different cohorts of patients. Taken together, our studies have demonstrated a previously undefined tumor suppressing role of CFTR and its potential as a prognosis indicator.

Biomarkers of Neonatal Infection and NEC – The State of the Art Approach

Pak C. Ng

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Despite advances in the management of preterm infants, neonatal infections and necrotizing enterocolitis (NEC) remain important causes of neonatal morbidity and mortality. Over one-fifth (21%) of very low birth weight (VLBW) infants have at least one episode of late-onset culture-proven sepsis. These infants require prolonged hospital stay and have significantly higher chances of developing bronchopulmonary dysplasia and adverse neurodevelopmental complications during infancy and early childhood. Preterm infants with NEC also have 3 fold increased risks of mortality compared with those who did not have the disease. However, early clinical features of infection and NEC are often subtle, nonspecific and difficult to recognize. Further, noninfected infants such as those with apnea of prematurity, acute exacerbation of chronic lung disease, functional gastrointestinal dysmotility and ileus are often clinically indistinguishable from infants who are in the early stages of sepsis or NEC. Hence, there is considerable interest in studying biomarkers of infection that can reliably differentiate between infected and noninfected infants. With increasing understanding of the inflammatory cascade of sepsis and rapid advances in diagnostic technologies, many potential infection markers have been investigated. We examine the research in this area and focus on major developments in recent years.

Recent research has led to the discovery of cell surface antigens, chemokines, cytokines and acute phase proteins that can potentially be used to 'rule in' or 'rule out' neonatal sepsis and sinister intra-abdominal pathologies. The diagnostic values of key inflammatory mediators, including neutrophil CD64, IL-6, IL-10, and IP-10, are promising and likely to become increasingly used as biomarkers for diagnostic and prognostic purposes. Multicolour flow cytometric analysis also has the advantage over immunoassay of being able to localise the activated biomarkers on a specific cell type, and the test can be performed on an *ad hoc* basis requiring only minimal volume of blood sample (0.05 mL whole blood). Our recent study also suggests that neutrophil CD64 is an excellent early warning biomarker for identification of patients with intra-abdominal sepsis/inflammation.

It is unlikely that a single compound can possess all the characteristics of an 'ideal' diagnostic biomarker. Serial measurements and use of combination of biomarkers have been reported to improve sensitivity and negative predictive value of these tests. Although current biomarkers are not infallible, judicious selection of a panel of mediators with complementary properties could greatly increase the ability of neonatal clinicians and microbiologists to diagnose infection and NEC, and discern valuable prognostic information. New techniques such as qPCR and proteomics studies may further discover important inflammatory mediators for diagnosis of sepsis and NEC.

Acknowledgement

Research grants were awarded by the Research Grant Council of the Government of Hong Kong, SAR (Project code: 4520-05M) and by the H.M. Lui Memorial Fund (Project code: 6901814) for carrying out the investigation of neutrophil CD64 in NEC and other markers in neonatal infection.

Age-dependent regulation of spermatogonial stem development by novel long non-coding RNAs

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Aging changes in the male reproductive system occur primarily in the testes, where spermatogenesis (sperm production) takes place. Spermatogenesis starts with undifferentiated Spermatogonial Stem Cell (SSC) populations, which are subsequently differentiated into spermatozoa (sperm) through meiotic division of spermatocytes and spermatids. Despite advances in genomics and molecular biology, previous attempts to delineate the developmental programs in SSC had limited successes, largely because the focus was limited to protein-coding genes from coding RNAs, which represent less than 10% of RNA transcripts. Long non-coding RNAs (lncRNAs) are non-protein-coding RNAs with more than 200 base pairs, and recently demonstrated to involve in developmental regulations in stem cell, brain and cancer. The role of lncRNAs in SSC maintenance and its regulation in advanced paternal aging are unknown. The aim of this project is to identify the function and regulation of long non-coding RNAs in the aging process of undifferentiated spermatogonial stem cell (SSC) population. Our central hypothesis is a subset of lncRNAs involves in SSCs aging regulation. We have identified an age-dependent lncRNA (*Age-lncRNA2*) exhibiting testis-specific and age-dependent expression. We aim to identify a set of Age-lncRNAs and delineate their functional role in SSCs. This research proposal is innovative because the effect of lncRNA biology in SSC aging has never been explored. The novel Age-lncRNAs candidates could lead to better understanding of advanced paternal age effect, in-vitro maintenance of SSC for preservation and fertility treatment. The findings are expected to substantially change the concepts of lncRNA function in developmental, reproductive and stem cell biology.

Angiotensin II-AT_2 receptor axis is involved in the functional maturation of human pancreatic progenitor cells toward the endocrine lineage

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Previous work has identified an islet RAS in the adult pancreas. Meanwhile the RAS regulates development of various stem/progenitor cells. Nevertheless, it has yet to be determined whether an RAS exists in the pancreatic progenitor cells (PPCs) and, if so, whether it is critical for the development of PPCs into functional islet-like cell clusters (ICCs), and their potential for islet transplantation.

To test this proof-of-principle hypothesis, we employed an established human fetal pancreas-derived PPC/ICC culture system to characterize the expression profile of RAS in PPCs and examine its effects on the differentiation of PPCs into ICCs. Results showed that RAS components were expressed and regulated throughout PPC differentiation, and that angiotensin II maintained PPC growth and differentiation via its AT_1 and AT_2 receptors. We also observed co-localization of AT_2 receptors with critical β-cell phenotype markers in PPCs/ICCs, as well as AT_2 receptor upregulation during differentiation. Interestingly, we found that AT_2, but not AT_1, receptor was essential for angiotensin II-induced upregulation of these β-cell development markers. Furthermore, lentivirus-mediated knockdown of AT_2 receptor suppressed the expression of these markers in ICCs. Transplantation of AT_2 receptor-depleted ICCs into immune-privileged diabetic mice failed to ameliorate hyperglycemia, implying that AT_2 receptors are critical during ICC maturation *in vivo*. These data indicate that an angiotensin II-AT_2 receptor axis is involved in regulating the functional maturation of PPCs toward the endocrine lineage.

Dab2 is a regulator of skeletal muscle development

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School of Biomedical Sciences, The Chinese University of Hong Kong.

Dab2 is an intracellular adaptor protein and a tumor suppressor. In mouse embryos, we found that Dab2 was first expressed in somites at E8.5, and then co-localized with muscle markers at E10.5. At advanced developmental stages, Dab2 was expressed in skeletal muscles. Based on these observations, we hypothesize that Dab2 plays essential roles in early skeletal muscle development.

To prove our hypothesis, C2C12 myoblasts and *Xenopus laevis* embryos were employed as *in vitro* and *in vivo* study models, respectively. *In vitro*, when C2C12 myoblasts were induced to differentiate into myotubes, Dab2 expression was increased, and Dab2 over-expression by full-length Dab2 transfection led to enhanced myotube formation. Conversely, suppression of Dab2 expression by Dab2 miRNA- or lentiviral shRNA-mediated knockdown resulted in reduced myotube formation. When Dab2 was re-expressed in myoblasts, myotube formation was partially restored. Microarray profiling on differentially expressed genes in normal myoblasts and myoblasts with Dab2 knockdown indicated that Dab2 was involved in regulating skeletal muscle development. *In vivo*, *XDab2* was found to co-localize with various muscle markers. Knockdown of *XDab2* expression with antisense morpholinos reduced expression of muscle markers.

In conclusion, our results demonstrate for the first time that Dab2 plays essential roles in the somite and skeletal muscle development.

Deletion of Dragon reveals the different roles of IL-6, TNF-α and IL-1β in inhibiting growth hormone signaling in the liver

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Protein hypercatabolism and muscle wasting characterize catabolic states induced by acute and chronic inflammatory diseases. The underlying mechanisms have not been completely elucidated. Evidence suggests that during systemic inflammation, the liver becomes resistant to growth hormone (GH) actions, leading to down-regulation of anabolic gene insulin-like growth factor-I (IGF-I), which activates the catabolic process. Increased production of proinflammatory cytokines IL-6, TNF-α and IL-1β has been implicated in the pathogenesis of hepatic GH resistance via potential mechanisms including down-regulation of the GH receptor (GHR), and up-regulation of suppressor of cytokine signaling-3 (SOCS3). However, the relative importance of and the detailed mechanisms for individual cytokines in regulating GH signaling are not fully understood.

In our previous study, we identified Dragon knockout mice as a chronic inflammation mouse model, which shows increased expression of a number of inflammatory factors including IL-6, TNF-α and IL-1β. Consistent with other mouse models, these mice showed down-regulation of GHR, up-regulation of SOCS3 and down-regulation of IGF-I in the liver compared to wilt-type mice. Neutralizing antibody to IL-6 did not alter GHR and IGF-I expression in the livers of Dragon knockout mice in comparison with control Dragon knockout mice while it reduced SOCS3 to basal levels. In contrast, neutralization of TNF-α and IL-1β in Dragon knockout mice increased GHR and IGF-I expression without significant effects on SOCS3 expression in the liver. Furthermore, double knockout of Dragon and IL-6 genes did not rescue retarded growth seen in Dragon knockout mice. Our *in vitro* studies demonstrated that TNF-α and IL-1β inhibited GHR expression but IL-6 had no effect in Huh7 human hepatoma cells. Interestingly, IL-6 was much more potent in stimulating SOCS3 expression than TNF-α and IL-1β. These results suggest that TNF-α and IL-1β play an important role in down-regulating IGF-I expression by inhibiting GHR expression in the liver. IL-6 up-regulates SOCS3 expression in the liver but this action does not play a significant role in inducing GH resistance when the components upstream to SOCS3 action in the GH signaling pathway are blocked.

Interaction between TRAPP and dynactin demonstrates a novel function of TRAPP in mammalian COPII vesicle tethering

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The transport of endoplasmic reticulum (ER)-derived COPII vesicles toward the ER-Golgi intermediate compartment (ERGIC) requires cytoplasmic dynein and is dependent on microtubules. To address whether and how COPII vesicle tether, TRAPP (Transport protein particle), plays a role in the interaction between COPII vesicle and microtubule, we discovered TRAPP subunit TRAPPC9 bound directly to dynactin subunit p150Glued via the same carboxyl terminal domain of p150Glued that binds Sec23 and Sec24. TRAPPC9 also inhibited the interaction between p150Glued and Sec23/Sec24 both in vitro and in vivo, suggesting that TRAPPC9 serves to uncouple p150Glued from the COPII coat, and to relay the vesicle-dynactin interaction at the target membrane. These findings provide a new perspective on the function of TRAPP as an adaptor between the ERGIC membrane and dynactin. By preserving the connection between dynactin and the tethered and/or fused vesicles, TRAPP allows nascent ERGIC to continue movement along the microtubule as they mature into the cis-Golgi.

Transcription factor Ets-1 plays dual roles in neural crest formation

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The neural crest (NC) is a transient embryonic cell population and found unique in vertebrate embryos. The NC population is highly migratory and pluripotency, and can differentiate into large variety of tissues including peripheral nervous system, craniofacial skeleton and melanocyte. It is induced at the border between prospective neural plate and epidermis. The neural crest cells subsequently undergo an epithelial-to-mesenchymal transition (EMT), delaminate from the neuroepithelium, and migrate to their final destinations, where they give rise to many derivatives.

Ets-1 belongs to the ETS family, which is a large family of transcription factors. Ets-1 plays essential roles during vasculogenesis and angiogenesis, and it is also involved in malignant transformation and tumor progression. Its roles in early embryonic development, however, are largely unknown. We identified ets-1 as one of downstream targets of Lrig3 during NC formation and differentiation. We found ets-1 is expressed in neural crest and its derivatives and its expression was regulated by FGF signaling. Ectopic expression of ets-1 repressed the expression of NC markers including *foxd3* and *snail2*. We found overexpression of ets-1 attenuated BMP signaling. Overexpression of ets-1 up-regulated neural markers, *sox2* and *zic1*; but down-regulated epidermis marker, *epidermis keratin*. Embryos injected with ets1MO showed inhibition of NC migration because of down-regulation of *adam13*. In line with this, we found overexpression of *ets-1* induced large expansion of adam13 expression domain. Our research revealed new roles of ets-1 in NC development, and shed light on the complexity of regulatory network that regulates NC formation.

A robust platform for efficient targeted gene disruption in zebrafish using TALENs

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Engineered nucleases are fusion constructs of the DNA binding domain of a transcriptional factor with the catalytic domain of fokI nuclease. The DNA binding domain brings the nuclease to a predetermined genomic locus to create DNA double-strand breaks (DSB). Repair of the DSB through the error-prone non-homologous end-joining (NHEJ) pathway leads to targeted gene disruption. Engineered zinc finger nucleases (ZFNs), created by fusion of zinc finger protein with fokI nuclease, have been developed as useful tools to knockout genes across species. However, because of the context-dependent nature of zinc finger proteins, generating effective ZFNs is labor intensive and time consuming. In addition, zinc finger proteins tend to bind to GNN (N representing any nucleotide) and some TNN triple bases, thus limiting the targetable sites of ZFNs in a genome. The transcription activator like effectors (TALEs) of plant pathogens seems able to overcome these limitations of zinc finger proteins. So far, transcription activator like effectors nucleases (TALENs) have been successfully used to modify genes in several species as well as in cell lines and human stem cells, with efficiencies comparable to the ZFNs. However, the full potentials of this novel TALENs approach are yet to be exploited. Here we describe a fast and robust platform for constructing highly effective TALENs to induce targeted mutations of miRNA- and protein-coding genes as well as targeted deletions of miRNA clusters in zebrafish. Our data indicated that TALENs is a convenient and powerful method for targeted gene knockout.

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 electrolytes[1]. 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 $P2Y_6$ receptor agonist, UDP, could stimulate transepithelial Cl^- secretion via both Ca^{2+}- and cAMP- dependent pathways[2]. In an established cell model of asthmatic inflammation[3], 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.

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Too much causes too little – a new teratogenic mechanism by retinoic acid

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Retinoic acid, an active metabolite of vitamin A, plays essential signalling roles in mammalian embryogenesis. Nevertheless, it has long been recognized that overexposure to vitamin A or retinoic acid causes widespread teratogenesis in rodents as well as humans. Although it has a short half-life, exposure to high levels of retinoic acid can disrupt development of yet-to-be formed organs, including the metanephros, the embryonic organ which normally differentiates into the mature kidney. Paradoxically, it is known that either an excess or a deficiency of retinoic acid results in similar malformations in some organs, including the mammalian kidney. Accordingly, we hypothesized that excess retinoic acid is teratogenic by inducing a longer-lasting, local retinoic acid deficiency. This idea was tested in an established in vivo mouse model in which exposure to excess retinoic acid well before metanephric rudiments exist leads to failure of kidney formation several days later. Results showed that exogenously applied retinoic acid caused prolonged downregulated expression of the retinoic acid synthesizing retinaldehyde dehydrogenase enzymes. Concomitantly, there was significant reduction in retinoic acid levels in whole embryos and kidney rudiments. Restoration of retinoic acid levels by maternal supplementation with low doses of retinoic acid following the teratogenic insult rescued metanephric kidney development and abrogated several extra-renal developmental defects. This previously undescribed and unsuspected mechanism provides a new insight into the molecular pathway of retinoic acid-induced teratogenesis.

Poster Presentation Session

4 June, 2012 (Monday) 14:00 – 15:00

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

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The metalloprotease ADAMTS8 displays anti-tumor properties through antagonizing EGFR/MEK/ERK signaling and is silenced in common carcinomas G.C.G. Choi, J. Li, Y. Wang, L. Li, B. Ma, J. Ying, B. Luo, W. Han, J. Yu, J.J. Sung, A.T.C. Chan, Q. Tao	P-03
Epigenetic disruption of Ras signalling through silencing of a Ras GTPase-activating protein RASA5 in human cancers Y. Fan, L. Li, L. Xiong, X. Su, A.T.C. Chan, Q. Tao	P-04
A novel protein translated from the human *KIAA0495* transcript at 1p36.3 is a pro-apoptotic tumor suppressor inhibiting oncogenic signaling and frequently silenced in carcinomas X.S. Shu, H. Geng, F.F. Poon, J. Peng, L. Soo, W. Wu, Y. Cheng, J. Ying, L. Wu, B. Ko, G. Srivastava, J. Yu, S.Y. Rha, H.K. Goh, A.T.C. Chan, J.J.Y. Sung, R.F. Ambinder, Q. Tao	P-05
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The therapeutic effect of Huperzine A on Alzheimer's disease involves a novel mechanism on brain iron regulation X. He, Q. Gong, M.S.C. Tam, Y. Ke	P-08
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Orange fluorescent protein: one protein, two structures? T.M. Ip, M.H. Chan, D.C.C. Wan	P-10
Characterization of miRNA-210 in regulating 3T3-L1 adipogensesis W.C. Liang, M.M.Y. Waye	P-11
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Do antidromic spikes jam the motor cortical output during therapeutic deep brain stimulation in parkinsonian rats? Q. Li, Y. Ke, D.Z.Y. Chan, H. Ko, W.H. Yung	P-13

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Screening for genes which can enhance reprogramming of human fibroblast into human iPSCs J.B. Hu, S.L. Kwan, B. Feng	P-15
Efficient directed differentiation of pluripotent stem cells to neural stem cells W.H. Tsang, X. Chen, W.Y. Chan, B. Feng	P-16
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Poster Presentations

Abstracts

The roles of microRNAs in tumor cell Epithelial Mesenchymal to Stem-Like Transitions (EMST)

H. Xia, M.C. Lin, H.F. Kung

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[Objectives] Epithelial-mesenchymal transition (EMT) is a crucial developmental program in which immotile epithelial cells acquire mesenchymal traits. Recently, several studies have demonstrated that EMT is associated with cells with stem cell properties. MicroRNAs, an abundant class of small ncRNA (non-coding RNA), have been shown to repress the expression of important cancer-related genes The objective or our study is to investigate the function and mechanism of miRNAs on EMT-associated generation of cancer stem-like cells.

[Methods] We examined the expression level of miR-200a and miR-124 in nasopharyngeal carcinoma (NPC) and glioma patient tissues and cell lines. By gain of function (over-expression) and loss of function (siRNA) studies we determine the effects of miRNAs in epithelial, mesenchymal and stem-like traits of the tumor cells.

[Results] We first showed that in NPC, stable knockdown of miR-200a promotes the transition of epithelial-like CNE-1 cells to mesenchymal phenotype. More importantly, it also induced several stem cell-like traits including CD133+ side population, sphere formation capacity, *in vivo* tumorigenicity in nude mice and stem cell markers expression. Consistently, stable overexpression of miR-200a switched mesenchymal-like C666-1 cells to epithelial state, accompanied by a significant reduction of stem-like cell features. In addition, *in vitro* differentiation of C666-1 tumorsphere resulted in diminished stem-like cell population and miR-200a induction. To investigate the molecular mechanism, we demonstrated that miR-200a controls EMT by targeting ZEB2, while it regulates the stem-like transition differentially and specifically by β-catenin signaling.
Furthermore, we showed that miR-124 is a brain-enriched miRNA that is downreguled in glioma. miR-124 inhibits glioma cell invasion, tumorigenicity and reduces neurosphere formation, CD133+ cell subpopulation and stem cell markers expression in part by targeting SNAI2.

[Conclusions] Our findings reveal for the first time the function of miR-200a and miR-124 in shifting NPC and glioma cell states via a reversible process coined as epithelial-mesenchymal to stem-like transition (EMST) through specific mechanisms.

Reversal of multidrug resistance by human cathelicidin LL37 and its fragments

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Human cathelicidin LL37 is an important class of endogenous antimicrobial peptides, which protects our body from invasive bacterial infection. Recently, several novel pharmacological uses of cathelicidin have been reported. Multidrug resistance (MDR) is an old but unresolved clinical problem hindering successful cancer chemotherapy. It is often mediated by the overexpression of ATP-binding cassette (ABC) drug efflux transporters on the surface of cancer cells, thus reducing intracellular concentration of anticancer drugs. The aim of this study is to investigate if LL37 and/or its fragments could inhibit these MDR transporters to help circumvent drug resistance. Human cancer cell line models with defined overexpression of the three major MDR transporters (i.e. P-gp, ABCG2 and MRP1) were used for cytotoxicity and drug efflux assays. Two short fragments of LL37 tested were found to potentiate the cytotoxic effect of mitoxantrone, an ABCG2 substrate anticancer drug, in cell lines with ABCG2 overexpression. The other two MDR transporters were not affected. The same LL37 fragments were also found to inhibit ABCG2-mediated drug efflux, which likely accounts for the MDR reversal effect. Interestingly, ABCG2 expression was also suppressed by the LL37 fragments, with a more pronounced suppression observed in the ABCG2-overexpressing drug resistant subline than in the parental cells. In summary, the LL37 fragments may represent promising lead compound for further development into useful MDR reversal agent for combination cancer chemotherapy.

The metalloprotease ADAMTS8 displays anti-tumor properties through antagonizing EGFR/MEK/ERK signaling and is silenced in common carcinomas

G.C.G. Choi, J. Li, Y. Wang, L. Li, B. Ma, J. Ying, B. Luo, W. Han, J. Yu, J.J. Sung, A.T.C. Chan, Q. Tao

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Disintegrins and metalloproteinases with thrombospondin motifs (ADAMTSs) have been reported dysregulated in various cancers, but their functional roles in tumorigenesis remain unclear. We refined a heterozygosity locus at 11q25 by array-CGH and identified ADAMTS8 as a novel candidate tumor suppressor gene (TSG). ADAMTS8 is broadly expressed in normal tissues but frequently downregulated or silenced by promoter methylation in multiple carcinoma cell lines, including nasopharyngeal (NPC), esophageal squamous cell (ESCC), gastric and colorectal (CRC) carcinomas. Pharmacological or genetic demethylation restored ADAMTS8 expression, indicating that promoter methylation directly mediates its silencing. Aberrant methylation of ADAMTS8 was further detected in several types of primary tumors but rarely in normal tissues. Ectopic expression of ADAMTS8 suppressed tumor cell clonogenicity through inducing apoptosis. We further found that ADAMTS8, as a secreted protease, inhibited EGFR/MEK/ERK signaling pathway, disrupted actin stress fiber organization and inhibited tumor cell motility. Thus, our data demonstrate that ADAMTS8 metalloprotease acts as a functional tumor suppressor through antagonizing EGFR/MEK/ERK signaling, in addition to its previously reported anti-angiogenesis function. Its frequent methylation in certain tumors could be developed as a potential biomarker.

Epigenetic disruption of Ras signalling through silencing of a Ras GTPase-activating protein RASA5 in human cancers

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Ras is a well-known oncogene. Aberrant mutations of *Ras* gene occur in approximately 30% of human tumors, causing constitutively activated Ras signaling. However, in certain types of tumors with *Ras* gene rarely mutated, abnormal Ras signaling pathway is still a common and critical event, suggesting alternative mechanisms for Ras signaling hyperactivation. Ras is active when it is bound to GTP, while the hydrolysis of bound GTP and inactivation of Ras is catalyzed by Ras GTPase activating proteins (RasGAPs). Using 1-Mb array CGH, we refined a small hemizygous deletion at 6p21.3 containing a RasGAP family member gene *RASA5*, which was named as *SynGAP* and studied only in the neuron systems. We demonstrated that *RASA5*, rather than other RasGAP family members *RASA2-4*, is broadly expressed in human normal tissues while epigenetically silenced in multiple tumors, especially in certain tumor types such as nasopharyngeal, esophageal and breast carcinomas with wild-type *Ras* but Ras cascade is constitutively active. Ectopic RASA5 expression led to growth inhibition, apoptosis, migration inhibition, as well as stem cell features repression of tumor cells. Meanwhile, knockdown of RASA5 through siRNA would benefit the tumor cell colony formation as well as epithelial-mesenchymal transition (EMT). RASA5 was shown to exert its tumor-suppressive activity through suppressing Ras-GTP level and further inactivating Ras signaling. Such an inhibitory effect could be partially abrogated in the presence of overexpressed dominant active Ras or by the deletion of the RasGAP domain of RASA5. For the first time, our study refined the role of RASA5 as a tumor suppressor, and demonstrated that the epigenetic silencing of this tumor suppressor represents a new mechanism of Ras activation in certain cancers with wild-type *Ras*.

A novel protein translated from the human *KIAA0495* transcript at 1p36.3 is a pro-apoptotic tumor suppressor inhibiting oncogenic signaling and frequently silenced in carcinomas

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Identification of tumor suppressor genes (TSG) elucidates oncogenic mechanisms and provides potential cancer biomarkers. Through integrative cancer genomics, we identified a novel 1p36.3 TSG candidate *TUSC6* (TUmor Suppressor Candidate 6, also known as *KIAA0495*), located immediately next to *TP73*. *TUSC6* is broadly expressed in human normal tissues, but silenced or downregulated in multiple tumor cell lines (nasopharyngeal, esophageal, gastric, and colorectal cancers), due to its promoter CpG methylation. However, no point mutation of *TUSC6* was detected in 20 cell lines examined. Aberrant *TUSC6* methylation was also frequently detected in multiple primary tumors, but seldom in normal tissues. The *TUSC6* transcript was validated to be protein-coding in both *in vivo* and *in vitro* expression systems. Moreover, endogenous expression of TUSC6 protein was observed in a panel of normal human tissues. TUSC6 is a nuclear protein which acts as a transcription repressor. The ectopic expression of TUSC6 in tumor cells lacking endogenous expression resulted in significant inhibition of anchorage-dependent and -independent cell growth mainly through inducing apoptosis, cell cycle arrest and senescence. TUSC6 inhibits oncogenic Akt, NF-κB and Wnt/β-catenin signalings through inhibiting Akt phosphorylation and promoting β-catenin degradation, leading to further tumor cell apoptosis. In conclusion, we have identified *TUSC6* as a novel 1p36.3 TSG, after *p73* and *CHD5*, being frequently inactivated by methylation in major tumors.

Dopamine related signaling pathways on generation of projection pattern at the mouse optic chiasm

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Ocular albinism is an inherited genetic disorder characterized by a lack of expression of melanogenic enzymes in the eye. Individuals with albinism show under-development of the fovea, reduced retinal cell number and misrouted ipsilateral axons at the optic chiasm. The rate-limiting enzyme in ocular melanin synthesis is tyrosinase, which oxidizes L-tyrosine to L-DOPA. L-DOPA is then decarboxylated into dopamine. Recent studies have shown that the protein product of ocular albinism type 1 gene, OA1, serves as a receptor for L-DOPA, forming part of dopamine related signaling system. In this study, we asked whether dopamine, L-DOPA and OA1 are expressed in early development of the mouse optic pathway, and determined whether interruption of dopamine signaling affects the routing of axons in the mouse optic chiasm. Using immunocytochemistry, we found L-DOPA, OA1, dopamine and its receptor D1A were expressed on the Vimentin positive neuroepithelial cells at embryonic Day (E) 13, and later (E14-15) on the retinal ganglion cells and their axons. In the chiasm, OA1, dopamine and D1A immunoreactivity was found on the RC2 positive radial glial cells at the midline. In vitro studies showed a dosage dependent inhibition of L-DOPA and dopamine to neurite outgrowth from E14 retina. The inhibitory effect of dopamine was alleviated by the presence of a D1A specific antagonist SCH23390. Furthermore, using a brain slice culture of the optic pathway, exogenous L-DOPA caused a reduction in axon crossing at the midline of E13 chiasm, when most axons are navigating through this region, but showed no obvious effect on the generation of uncrossed pathway in E15 preparations. We concluded that L-DOPA and dopamine are negative regulators to optic axon growth, probably through binding to OA1 and D1A, respectively. Moreover, these dopamine signaling related molecules may play a role on axon crossing during early development of the pathway, but not on bilateral routing that develops at later stages of development.

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Suramin is a potent stimulator of retinal ganglion cell regeneration after optic nerve injury

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Purpose

Suramin, an anti-parasitic drug and a P2 purinergic receptor antagonist, has been reported to be neuroprotective. However, whether suramin supports regeneration of the central nervous system is not known. We asked whether suramin promotes the regeneration of retinal ganglion cell (RGC) axons after optic nerve (ON) injury.

Methods

RGC axons of adult hamsters were damaged by cutting the ON. Suramin or its vehicle (saline) was injected intravitreally after ON injury. At 1 week post-ON cut, RGC survival was quantified by βIII-tubulin immunostaining, and the proportion of surviving RGCs that expressed the growth-associated protein GAP-43, a marker that correlates with regenerative propensity, was determined. Peripheral nerve (PN) grafting to the cut ON was used to assess whether suramin boosted the regeneration of RGC axons into the graft. The potency of suramin on RGC regeneration was compared against ciliary neurotrophic factor (CNTF, a growth factor well known to activate RGC regeneration), PPADS (a broad range P2 antagonist), or suramin analogues (with specificity towards selective P2 subtypes).

Results

Both suramin and CNTF promoted RGC survival compared to the vehicle control. Suramin was also a strong promoter of RGC regeneration, as shown by the dramatic increase in the number of GAP-43-expressing and regenerating RGCs (4 and 8 times of the vehicle control respectively). It was even more effective than CNTF on RGC regeneration. In contrast, PPADS did not enhance GAP-43 expression and regeneration of RGCs above that of vehicle control, suggesting that suramin promoted regeneration independent of P2 antagonisation. This was also reflected in studies with suramin analogues in which no clear-cut pattern was seen: NF157 and NF279 promoted GAP-43 expression and regeneration similar to suramin, while NF023 and NF110 resembled the control.

Conclusions

Suramin promotes RGC axonal regeneration better than CNTF. The mechanism involved may be distinct from P2 blockade.

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The therapeutic effect of Huperzine A on Alzheimer's disease involves a novel mechanism on brain iron regulation

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The accumulation and deposition of amyloid-β (Aβ) peptides in the brain is a central pathological hallmark in Alzheimer's disease (AD). Recent studies suggest a role of iron accumulation in the brain in the pathogenesis of AD. Huperzine A (HupA), a new alkaloid isolated from the Chinese herb *Huperzia serrata*, is a potent and reversible inhibitor of acetylcholinesterase. HupA is neuroprotective and improves cognitive functions. In this study, we found that the beneficial effect of HupA is related to a novel action on brain iron regulation. APPsw/PS1dE9 transgenic AD mice were treated with HupA (0.1mg/kg, po, once per day) or saline. We found that HupA treatment reduced insoluble but not soluble Aβ levels and markedly ameliorated amyloid plaques formation in these animals at 9th month of age. These effects of HupA were reduced by feeding the animals with a high iron diet starting from the second month. In parallel, we found that HupA decreases iron content in the brain. This effect was probably related to the action of HupA in decreasing the expression of transferrin-receptor 1 (TfR1) leading to reduced transferrin-bound iron uptake into cultured neurons. Taken together, these results suggest that, in addition to facilitated cholinergic transmission and neuroprotection, regulation of iron metabolism is a novel target of HupA in its therapeutic action on dementia.

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The new selective NK_1 receptor antagonist, Netupitant (NETU), with Palonosetron (PALO) and Dexamethasone (DEX), reduces Cisplatin-induced acute and delayed emesis

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The prevention of chemotherapy-induced acute and delayed emesis often involves the use of older generation anti-emetics where the multiple dosing frequency and efficacy is not ideal. In the present studies, we investigate the anti-emetic potential of a single administration of PALO and NETU, alone with DEX, and combined all together, in the cisplatin-induced acute and delayed emesis model in the ferret.

Male ferrets were administered PALO 0.03-0.1 mg/kg and/or NETU 0.1-1 mg/kg orally 15 min before cisplatin 5 mg/kg, i.p.; DEX 1 mg/kg, i.p. was administered 15 sec before cisplatin and then continued at 24 h intervals. Food intake and retching and/or vomiting were recorded for up to 72 h.

Cisplatin induced 205.6±40.5 and 471.0±98.3 retches+vomits during the 0-24 (acute) and 24-72 h (delayed) periods, respectively. PALO and NETU alone with DEX, dose-dependently reduced acute emesis. Combination of all drugs enhanced the antiemetic activity. At the highest doses, the reduction of acute emesis by PALO plus NETU plus DEX was almost complete (99 %, $P<0.01$); delayed emesis was reduced by 60 % ($P<0.05$). PALO alone at 0.1 mg/kg with DEX, and in combination with NETU 1 mg/kg and DEX, significantly antagonized the cisplatin-induced reduction in feeding by 41 ($P<0.05$) and 38 % ($P<0.05$), respectively.

The single oral regimen of PALO and/or NETU combined with DEX is highly effective in antagonizing cisplatin-induced acute and delayed emesis. The ability of the combination to also antagonize cisplatin-induced decreases in food intake suggests a positive effect on appetite that may relate to an ability to reduce nausea.

Orange fluorescent protein: o**ne Protein, two structures?**

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Native Orange Fluorescent Protein (OFP) is a tetrameric GFP-like protein cloned from *Cnidarian* tube anemone *Cerianthus* sp. To make it a favorable imaging tool for research use, it was mutagenized to monomeric form, designated mOFP. The emission spectrum of purified mOFP reveals that the observed orange fluorescence contains two emission maxima, one green and one red, peaked at 510 and 575 nm respectively. Surprisingly, during further purification process, it was observed that mOFP actually contained two populations of fluorescent species. Mass spectrometry analysis confirmed that these two species were having identical underlying protein sequence but different chromophore configurations, probably due to different post-translational modifications. One species is a green fluorescent mOFP (G-type mOFP) having GFP-like chromophore structure with sp^3 C found in the chromophore tri-peptide and the other one is a red fluorescent mOFP (R-type mOFP) having DsRed-like chromophore structure with sp^2 C instead. This unique feature of mOFP provides us with insight the evolution of green to red fluorescent proteins. This is the first report of a fluorescent protein co-expressing with two types of chromophore during protein maturation but sharing an identical primary sequence.

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Characterization of miRNA-210 in regulating 3T3-L1 adipogensesis

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MiRNAs, 20 to 25 nucleotides in length, represent a class of naturally occurring small non-coding RNA molecules. They regulate gene expression at the post-transcriptional level and hence control cellular mechanisms including developmental transitions, organ morphology, differentiation and cell proliferation. Although growing evidences indicate that miRNAs are involved in cell growth and differentiation, how they contribute to the process of adipocyte differentiation remains elusive. In the present study, we revealed that the expression level of miR-210 was significantly upregulated during adipogenesis. Ectopic introduction of miR-210 into 3T3-L1 cells promoted clonal expansion as well as terminal differentiation. Western blotting results demonstrated that overexpression of miR-210 in 3T3-L1 cells provoked adipocyte differentiation via activation of PI3K/Akt pathway. Together, we have identified miR-210 as an important positive regulator in adipocyte differentiation through the activation of PI3K/Akt pathway.

The regulation of glial cell activity by dorsal root ganglion neurons

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In the intact dorsal root ganglion (DRG), increased expression of glial fibrillary acidic protein (GFAP) by satellite glial cells is taken as evidence of glial cell activation, and is readily identifiable following nerve injury. Expression of glutamine synthetase (GS) allows detection of satellite glial cells in non-stimulated DRG. Using real time RT-PCR, we have detected increased GFAP mRNA expression in freshly prepared DRG cells compared with intact DRG. GFAP mRNA levels fell after 24 h in culture and then surprisingly increased again after a further 3 days. In contrast, there was a progressive loss of GS mRNA expression in isolated rat DRG cell cultures containing both neurons and glial cells, and this loss was greatest in the absence of neurons. Incubating mixed DRG cells with a range of concentrations of glutamate indicated that 10 mM glutamate significantly increased GS mRNA expression ($P<0.05$) without evidence of neurotoxicity. By better understanding the factors regulating GFAP and GS expression *in vitro*, we hope to gain insights into the crosstalk between DRG neurons and satellite glial cells in intact DRG. (This work was supported by a grant from the Research Grants Council of the Hong Kong SAR (GRF476710)).

Do antidromic spikes jam the motor cortical output during therapeutic deep brain stimulation in parkinsonian rats?

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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 cortex from the STN has been hypothesized to contribute to the DBS effect but has not been demonstrated in freely moving animals and its mechanism remains obscure. Here, in the freely moving hemi-parkinsonian rats, we identified short latency antidromic spikes in layer V motor cortical neurons during STN-DBS. Increased failure rate with increasing stimulation frequency produced the highest frequency of random antidromic spikes at 125 Hz stimulation, which correlated with the optimal therapeutic efficacy. This effect was accompanied by increased firing rate, reduced burst spiking and synchrony of firing in the motor cortical projection neurons. Field potential analysis revealed normalization of the pathological beta rhythm and spike-field coherence. Importantly, we found evidence that the firing probability of the cortical projection neuron is modified following the occurrence an antidromic spike suggesting that direct interference of synchronized firing by stochastic antidromic spikes underlies the beneficial effect of DBS in PD. Our results therefore support that STN-DBS antidromically activates output neurons in the motor cortex through the corticosubthalamic nucleus projection, which directly disrupts abnormal neural activities in the motor cortex in PD.

Reprogramming MSCS through *in vitro* differentiation and dedifferentation for enhancing therapeutic potential *in vivo*

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Successful treatment of human glioma, the most deadly brain tumor, has not yet been achieved largely due to deficiencies in delivery strategies for therapeutic agents. Recently, Mesenchymal Stem Cells (MSCs)-mediated gene delivery has been shown to be a promising strategy for improving the efficacy, and minimizing the toxicity, of gene therapy in the treatment of glioma. However, low levels of MSC recruitment and persistence *in vivo* largely limit their overall effectiveness and clinical use. Therefore, development of methods for improving the homing of MSCs into tumor microenvironment, and increasing therapeutic effects of the gene is in burning need. Interestingly, we have demonstrated that after *in vitro* induction of neuronal differentiation and dedifferentiation, MSCs, which have already committed to neuronal lineage, revert to a primitive cell population (dedifferentiated MSCs, De-MSCs) retaining stem cell characteristics but exhibiting a reprogrammed phenotype distinct from naïve MSCs. Of therapeutic interest, De -MSCs exhibit enhanced cell engraftment compared to unmanipulated MSCs *in vivo*, with significantly improved cognition function in a neonatal hypoxic-ischemic brain damage (HIBD) rat model. More interestingly, we have revealed that De-MSCs express significantly higher levels of chemokines and cytokines, i.e. SDF-1/CXCR4 and TNFα, and display enhanced tropism to cancer, indicating De-MSCs might be of therapeutic values in the treatment of glioma. On the basis of these findings, further studies aiming to achieve a better tumor targeting and gene delivery system for glioma therapy are underway.

Screening for genes which can enhance reprogramming of human fibroblast into human iPSCs

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Reprogramming of somatic cells into induced pluripotent stem cells (iPSC) has opened tremendous opportunities for regenerative medicine. However, further clinical application of iPSC technique has been hindered by the shortcomings of the current reprogramming methodologies, especially the inefficiency. To overcome this limitation, screening for factors that can trigger rapid, efficient and safe reprogramming is highly demanded.

In this study, we aim to perform a systematic screening to identify novel factors that can promote the reprogramming of human somatic cells, for the purpose of improving efficiency and generating human iPSCs in a shorter course of time. The to-be screened genes are selected according to their function in the maintenance of ESCs as well as their pluripotency-associated expression. The reprogramming potential of candidate genes will be examined and their effect will be quantified by the formation of iPSC colonies. The iPSCs induced by candidate factors will be analyzed from the aspect of their expression profile, epigenetic status and differentiation capacity by comparing to human ESCs. The most efficient candidates will be further investigated for their functional roles in controlling pluripotency, using, for example, the genome-wide location analysis approach.

Efficient directed differentiation of pluripotent stem cells to neural stem cells

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Derivation of neural stem cells (NSC) from induced pluripotent stem cells (iPSCs) provides an unlimited cell source for the potential regenerative medicine for traumatic CNS injury and neural disorders. However, a standardized methodology for the efficient differentiation of iPSC to a homogeneous NSC population is currently lacking. Here, we attempted to differentiate iPSC to neural fate through modulating extracellular signals. For the purpose of prove principle, mouse pluripotent ES cells and iPSCs are used as models. We have set up the culture conditions for mouse NSC, as well as induction condition for further differentiating these cells into other neuronal cells, such as neurons, astrocytes and oligodentrocytes. Based on these settings, we investigate the differentiation of PSC and found that the efficiency of induction from PSC to NSC is enhanced by the modulation of Transforming growth factor β (TGF-β) and Wnt signaling, and clonally derived neurospheres composing primitive NSC (pNSC) could generate more committed definitive NSC (dNSC). The derived NSC are characterized according to their expression profile, differentiation potential and tumorigenicity. The neuronal differentiation potential would be studied both in vitro and in vivo by marker expression, while the integrating ability and tumorigenicity of derived NSC could be studied from in vivo transplantation.

Co-localization of BRE (TNFRSFA1 modulator/BRCC45) with Oct4 in mouse embryos and its possible role in stem cell and embryo development

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The brain and reproductive organ-expressed protein (BRE) gene encodes a highly conserved protein found in a number of species including human and rodents. The function of BRE in mouse embryogenesis and stem cells has not been elucidated. Here we reported that BRE expression is detected ubiquitously in most fetal organs including: brain, liver, heart, lung, thymus, limb and chondrocytes in ribs-as revealed by RT-PCR and/or immunohistochemistry. Immunofluorescent staining of embryo sections showed that there was increased BRE expression in the heart and epithelial lining of main bronchus of the developing lung bud, where expression of Oct4 was also detected. These results indicated that BRE and Oct4 are precisely co-regulated in embryo development, suggesting that they play vital roles in organogenesis. Co-expression of BRE and Oct4 was also detected in spermatogonia and primary spermatocyte inside adult mouse testis and mouse embryonic stem (ES) cells. In addition, we found that upon differentiation, expression of BRE in ES cells was down-regulated. The above results indicated that BRE plays important roles in stem cell and embryonic development, and that Oct4 and BRE expression is directly or indirectly inter-related in maintaining stem cell pluipotency. We generated a BRE promoter-reporter construct by inserting a BRE proximal promoter followed by a LacZ reporter gene. Overexpression of Oct4 in NIH3T3 cells by transient transfection leads to activation of BRE promoter reporter, suggesting that Oct4 can activate BRE gene expression and the transcriptional activation is mediated by BRE promoter. BRE may act as one of the down-stream targets of Oct4.

The role of cellular retinol binding protein 1 (CRBP1) in regulating function of mesenchymal stem cells (MSCs)

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Introduction: CRBP1 (cellular retinol binding protein 1), takes part in vitamin A metabolism and intracellular transporting of retinoid. The current studies investigate the roles of CRBP1 in MSCs multi-differentiation potential and migration ability.

Methods: Cultures of rat BM-MSCs were established; the gene encoding rat CRBP1, β-catenin and RXRα were amplified and cloned separately. Two different shRNAs were chosen from rat CRBP1 mRNA sequence. MSCs were then transduced with lentivirus carrying CRBP1, dsRed, or shRNAs and the transduction efficiency was checked by RT-PCR and western blot. Osteogenic and adipogenic differentiation were performed according to the published protocols.

Results: CRBP1 was successfully transduced into MSCs with more than 80% of cells were positive for CRBP1. CRBP1 overexpression could enhance osteogenic differentiation of MSCs, while inhibit their adipogenic differentiation, as demonstrated by quantitative RT-PCR (ALP, Runx2, OCN, OPN, Col1α2), western blot (OPN), Alizarin Red S or Oil Red O staining and ectopic bone formation. When endogenous CRBP1 was knocked down, the effect of CRBP1 on osteogenesis and adipogenesis was reversed. Furthermore, we showed that CRBP1 may have promoted osteogenic differentiation of MSCs through inhibiting RXRα-induced β-catenin degradation, maintaining β-catenin and pErk1/2 at higher levels. CRBP1 overexpression had no significant effect on migration of BM-MSCs. Finally, the effect of CRBP1 on osteogenesis and adipogenesis of BM-MSCs was confirmed in vivo by ectopic bone formation carried out in nude mice.

Discussion: We showed that over-expression of CRBP1 promoted osteogenic differentiation and inhibited adipogenic differentiation of BM-MSCs in vitro and in vivo. Furthermore, we demonstrated that CRBP1 promotes osteogenic differentiation of MSCs through inhibiting RXRα-induced β-catenin degradation, maintaining β-catenin and pErk1/2 at higher levels. Taken these data together, our study shows that CRBP1, apart from its known function, also plays important roles in regulating osteogenesis and adipogenesis of MSCs at least partially through β-catenin and Erk1/2 signaling pathways.

Automated quantitative gait analysis in mouse model of Parkinson's disease

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Gait and postural deficits are important features in Parkinson's disease (PD) patient. Current behavioral tests in C57BL/6 mice model of PD induced by methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP) only determine approximately akinesia and dyskinesia, data on gait and posture is lacking.

Our research group first documented gait and postural changes using the computer-assisted Catwalk system. In order to screen the sensitive parameters, we further correlated these with the expression of tyrosine hydroxylase (TH) protein in C57BL/6 mice model of PD induced by methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).

The results showed that walking speed of PD mice was significantly decreased after MPTP injection. In gait analysis, maximum intensity and swing duration in all limbs and limbs were both reduced while those of stride length, swing speed, and stance were increased in the fore limbs but decreased in the hind limbs. In posture analysis, the index for base of support (BOS) was increased after administration of MPTP, while the indices for maximum contact area and print length in the four paws, and print width and print area in the hind paws were decreased. To correlate these functional deficits with TH protein levels in the substantia nigra, we selected some predictive indicators for analysis. BOS, stride length, swing speed, and maximum intensity in the hind limbs correlated more closely with TH level than distance in the open-field test.

It is conclude that gait and postural analysis using the Catwalk system provides reliable and sensible criteria to investigate gait and postural changes, caused by MPTP-induced systemic lesions in C57/BL6 mice, and could be an effective and novel tool for screening new drugs or neuroprotective gene strategies.

Hedgehog signaling interacts with Hippo pathway in the development of osteosarcoma

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Osteosarcoma (OS) is one of the most common primary bone tumors and is frequently found in adolescence. However, little is known about the genetic cause and pathogenesis of OS and its prognosis remains poor. Hedgehog (Hh) signaling and Hippo signaling have been widely implicated in the development of many cancers and previous studies showed that these two pathways interact in medulloblastoma. Our previous data showed that cell autonomous upregulation of Hh signaling in mature osteoblasts leads to increased bone formation. Thus, we reasoned that deregulation of Hh signaling may be one of the risk factors in the development of OS. Here, we generated an osteosarcoma mouse model, *Ptch*$^{c/+}$*; p53*$^{+/-}$*; HOC-Cre*, in which Hh signaling is partially upregulated in mature osteoblasts in a *p53*$^{+/-}$ background to enhance the incident rate of OS. Ubiquitous upregulation of Hh signaling in osteoblasts (*Ptch*$^{c/c}$*; HOC-Cre*) is early lethal, which prohibits the development of osteosarcoma. Our results demonstrated that the *Ptch*$^{c/+}$*; p53*$^{+/-}$*; HOC-Cre* mutant developed OS at a relatively high frequency, starting from 7-8 months. Primary OS cancer cell lines derived from this mouse models showed upregulated *Gli1* and *Gli2* expression, the major effectors of Hh signaling. More interestingly, the main transactivator of the Hippo pathway, *Yap* (*yes-associated protein*) is also upregulated. Similar expression patterns were also found in the *Ptch*$^{c/c}$*; HOC-Cre* calvaria tissues, suggested that this interaction is p53-independent. Hh agonist, SAG, and antagonist, cyclopamine, activated and suppressed Hippo signaling in MC3T3-E1 osteoblastic cell lines, respectively, as revealed by dual luciferase assays. More importantly, the downstream target genes of Hippo pathway, *Ctgf* and *Cyr61*, were also upregulated in both OS cell lines and calvaria tissues of the *Ptch*$^{c/c}$*; HOC-Cre* mutant mice. These results strongly suggest that Hh signaling interacts with Hippo pathway in the development and pathogenesis of OS.

Screening of biomarkers of global cerebral ischemia

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Stroke is one of the most common causes of death worldwide, and remains a heavy burden on communities and healthcare systems. Global cerebral ischaemia is one of the most prevalent types of stroke, with cardiac arrest it's most common cause. The latter produces cell injury and death in hippocampus, which are selectively vulnerable.

The two-vessel transient global cerebral ischaemia model provides a simple and stable platform to understand the mechanism of brain injury and evaluate the efficiency of therapeutic strategies. However, a systematic study and quantification of biomarkers have not been conducted. This study aims to establish a stable mouse model of transient global cerebral ischaemia and to screen biomarkers related to its inflammatory and recovery phases.

To screen biomarkers after cerebral ischaemia in the inflammatory phase and recovery phase, various quantitative assays were carried out. First, two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) was used to detect the unknown changes in protein levels, then a reverse transcription polymerase chain reaction (RT-PCR) to detect changes in mRNA level, and finally western blot to confirm the changes in protein expression levels of the markers. We screened synapsin II, neurofilament medium protein (NF-M) and heat-shock protein 70 (HSP-70) as biomarkers. Those markers hold the potential usages as therapeutic targets.

Deletion of insulin receptor in chondrocytes sensitizes IGF-1 signaling and action

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Insulin/insulin receptor (IR) signaling is central to regulating energy and glucose metabolism, growth and body composition. Insufficient insulin production or action associated with diabetic states is accompanied with impaired bone healing. IR and IGF-1 receptor (IGF-1R) exist in the cartilage, and IGF-1 stimulates growth plate development. However, the role of IR in chondrocytes remains unclear. This study aims to investigate the function of insulin/IR signaling in chondrogenesis during skeletal growth. The Cre-loxp strategy was used to delete the IR specifically in chondrocytes in vitro and in vivo. We found that insulin treatment increased the total length of metatarsals over that of untreated controls, characterized by increased size of the proliferating zone and cellular proliferation indexed by the number of proliferating cell nuclear antigen immunoreactive cells. Deletion of IR elevated IGF-1R mRNA and protein levels. IGF-1 stimulated Akt and ERK phosphorylation in the control cells, and the effects were enhanced in the IR mutant cells. Deletion of IR decreased chondrogenic differentiation and proteoglycan synthesis, and the effects were rescued by IGF-1. In vivo phenotyping showed that the cell numbers in the proliferating zone of the growth plate were increased and the cell sizes were smaller in mice lacking IR in chondrocytes compared with control littermates though the length of the growth plate was maintained. The data suggests that deletion of IR in chondrocytes sensitizes IGF-1 signaling and action as a function of compensation.

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AVE3085 restores endothelial function in type 2 diabetic *db/db* mice through enhancement of eNOS expression

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Background Reduced nitric oxide (NO) bioavailability is a hallmark of diabetes-related vascular complications. This study investigated whether enhancement of endothelial NO synthase (eNOS) expression by AVE3085 can improve endothelial function in type-2 diabetic db/db mouse.

Methods Aortae from C57BL/6J mice were cultured with high glucose (30 mM) and AVE3085 (1 μM) for 48 h. Twelve-week-old db/db mice were orally administrated with AVE3085 (10 mg/kg/day) for 7 days. Vascular reactivity was assessed in isometric and isobaric myography. Reactive oxygen species (ROS) levels and NO level were detected with fluorescence dyes. Protein expression was detected by Western blotting.

Results AVE3085 prevented the high glucose-induced impairment of endothelial function in mouse aortae and potentiated the NO production in cultured endothelial cells. Chronic treatment with AVE3085 improved endothelium-dependent relaxations in aortae, mesenteric and renal arteries of *db/db* mice; and enhanced flow-mediated dilatation in mesenteric arteries, accompanied by reduced ROS levels and increased eNOS expression.

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

Protective effects of N-acetyl-L-cysteine in pancreatic islets against hyperglycemia-induced oxidative stress

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This study was designed to elucidate the mechanisms of action involved of N-acetyl-L-cysteine (L-NAC) on the restoration of the blunted functions of pancreatic islets caused by hyperglycemia. Isolated pancreatic islets and single pancreatic β-cells of lean (db^+/m^+) and obese/diabetic (db^+/db^+) mice were used. Effects of L-NAC (20 mM, 24 hr) on mitochondrial H_2O_2 levels, F-actin cytoskeleton expression, glucose-induced insulin secretion (GIIS) and insulin content of the pancreatic islets of both strains of mice were evaluated. GIIS and insulin content of isolated pancreatic islets were lowered in db^+/db^+ mice compared with db^+/m^+ mice. The blunted GIIS and suppressed insulin content of isolated pancreatic islets of db^+/db^+ mice were partially restored by L-NAC. Elevated glucose challenge (15 mM) resulted in a higher mitochondrial H_2O_2 level (L-NAC sensitive) in pancreatic β-cells of db^+/db^+ mice. Cortical F-actin cytoskeleton expression in db^+/db^+ mice was higher and it was attenuated by L-NAC. Our results demonstrated that *ex vivo* L-NAC restored the blunted GIIS and the reduced insulin content of pancreatic islets in db^+/db^+ mice. Therefore, we propose the reduction of mitochondrial H_2O_2 generation and cortical F-actin cytoskeleton expression as possible therapeutic strategies for treating diabetes mellitus.

Evidence for involvement of potassium and calcium channels in the cerebral vascular effects of Danshen and Gegen

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Danshen (D) and Gegen (G) are used in traditional Chinese medicine for treatment of cardiovascular diseases. In this study, we have investigated their effects individually and in a combined DG formation (ratio 7:3) on rat-isolated cerebral basilar artery, and determined the significance of potassium and calcium channels in mediating their effects.

Effects of Danshen, Gegen, and the DG formulation on tension responses of the rat-isolated basilar arteries were investigated using a standard myograph system. Patch-clamp and con-focal studies were performed on smooth muscle cells isolated from rat basilar arteries to investigate their effects on K_{ATP} and Ca^{2+} channels, respectively.

Danshen, Gegen, and the combined DG formulation all produced concentration-dependent relaxation of the rat basilar arteries. Their effects were not affected by 4-aminopyridine (K_v blocker), barium chloride (K_{IR} blocker), or iberiotoxin (BK_{Ca} blocker), but were partially inhibited by glibenclamide (K_{ATP} blocker), tetraethylammonium (non-specific K^+ blocker), and by a combination of all these K^+ channel inhibitors. Patch-clamp studies also showed that these agents produced enhancement of K_{ATP} currents in the vascular smooth muscle cells, and con-focal microscopic studies showed that they inhibited Ca^{2+} influx. These agents also produced dose-dependent inhibition on $CaCl_2$-induced contraction in tension studies.

Danshen and Gegen, used individually or as a combined formulation, were capable of relaxing cerebral blood vessels. Both tension and electrophysiological findings confirmed that their effects were produced by inhibition of Ca^{2+} influx into the vascular smooth muscle cells; part of which was mediated by the opening of K_{ATP} channels. The cerebral vasodilator activities of these agents might benefit patients with obstructive cerebrovascular diseases.

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

Functional role of TRPV4-$SK_{Ca}3$ coupling in vascular endothelial cells in normal and streptozotocin-induced diabetic rats

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Two populations of endothelial cell K^+ channels, the small conductance and intermediate conductance Ca^{2+}-activated K^+ channels (SK_{Ca} and IK_{Ca}), are known to be involved in the endothelium-dependent hyperpolarization. Ca^{2+} entry into endothelial cells stimulates these channels, causing membrane hyperpolarization of endothelial cells and underlying smooth muscle cells. In the present study, we investigated the interaction of $SK_{Ca}3$ with a Ca^{2+} entry channel TRPV4 in endothelial cells and explored the functional role of such interaction in normal and streptozotocin-induced diabetic rats. Co-immunoprecipitation and sub-cellular co-localization experiments showed that TRPV4 and $SK_{Ca}3$ bind to each other to form a physical complex, allowing efficient signal transduction between these two molecules. In the primary cultured vascular cells and isolated mesenteric artery segments, stimulation of TRPV4 by acetylcholine induced Ca^{2+} entry, which activated $SK_{Ca}3$ and cause consequent smooth muscle hyperpolarization and vascular relaxation. In anesthetized rats, the TRPV4-$SK_{Ca}3$ pathway contributed to the regulation of body blood pressure and local blood flow in mesenteric beds. In streptozotocin-induced diabetic rats, TRPV4-$SK_{Ca}3$ coupling was impaired, which could be the underlying reason for EDHF dysfunction in these animals. These results demonstrated an important physiological and pathological role of TRPV4-$SK_{Ca}3$ coupling in vascular endothelial cells.

Novel molecular targeted therapy for glioblastoma

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Glioblastoma is a common malignant brain tumour. Although the patients with glioblastoma always receive multi-modality treatment, the prognosis remains poor. Glioblastoma is resistant to conventional cancer therapy may be due to its diffuse and infiltrative nature. Recently, the tumour necrosis factor-related apoptosis inducing ligand (TRAIL) has been shown to be responsive to in experimental solid cancers such as colon, lung, and breast cancer. It induces cell apoptosis through connecting with death receptors. In this work, we examine the potential roles of TRAIL in the treatment of glioblastoma.

The results showed that two out of three human glioblastoma cell lines are resistant to TRAIL-induced apoptosis. However, lovastatin, which is a 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitor, has been shown to sensitise glioblastoma cells to TRAIL-induced cell death via up-regulation of DR5 (TRAIL-R2) expression. The further experiment also confirmed that the up-regulation of DR5 might relate to the NF-κB pathway and the NF-κB p65 plays a key role in this phenomenon.

Furthermore, the effect of lovastatin and TRAIL was also evaluated in a nude mice subcutaneous brain tumour model. We found that the DR5 expression of tumour tissue was markedly up-regulated in tumour-bearing mice with local peri-tumoural administration of lovastatin and the consequent study also confirmed that local peri-tumoral injection of lovastatin and TRAIL significantly decreased tumour volume in subcutaneous nude mice model. These data give us a new insight in the targeting therapy for glibolastoma and DR5 is a potential biomarker for screening glioma patients who are sensitive to TRAIL therapy.

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Generation of tendon progenitor cell line from tendon-derived stem cells (TDSCs) by overexpression of scleraxis

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Scleraxis (Scx) is a bHLH transcription factor that is expressed in the early stage of tendon development. Scx was reported to positively regulate the expression of collagen type 1, which encodes the most abundant protein in tendon, via the tendon-specific element 2 of the procollagen type 1, alpha 1 (Col1a1) promoter. Scx knock-out mice demonstrated significant impaired formation of load-bearing tendons. Thus, Scx is a key regulator of tendon differentiation. Transplantation of BMSCs over-expressing Scx improved rotator cuff healing in a rat model. Over-expression of Scx led to tenogenic differentiation of immortalized human BMSCs in vitro. However, the tenogenic effect of Scx overexpression in tendon-derived stem cells (TDSCs) has not been reported. In our previous study, TDSCs showed higher colongenicity, proliferative and multi-lineage differentiation potentials compared to BMSCs and might be a better cell source for tendon repair. This study thus aimed to investigate the effect of over-expression of Scx in the tenogenic differentiation of TDSCs. Scx-dsRed plasmid was constructed and transduced into rat GFP-TDSCs using lentiviral system. GFP-TDSCs-Scx line was successfully established. The GFP-TDSCs-Scx line showed significantly higher Scx mRNA expression compared to wild-type GFP-TDSCs and GFP-TDSCs-mock. GFP-TDSCs-Scx expressed significantly higher mRNA level of tenogenic markers including Col1a1, Thbs4, Tnc and lower mRNA level of chondrogenic marker Acan compared to GFP-TDSC-mock. However, overexpression of Scx in GFP-TDSCs has no effect on the expression of Tnmd. In conclusion, tengoenic progenitor cell line was generated from TDSCs by overexpression of Scx. The effect of this tendon progenitor cell line compared to undifferentiated TDSCs in tendon repair will be compared in the future.

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The role of erythropoietin and erythropoietin receptor in chondrogenesis

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Erythropoietin (EPO) and EPO receptor (EPOR) are essential cytokine signals that control the proliferation, differentiation and survival of erythroid progenitors and the production of red blood cells. In addition, EPO/EPOR signalling involves in the development and regeneration of several non-hematopoietic organs including heart, brain, and bone, et al. It is shown that EPO promotes differentiation of mesenchymal stem cells into osteoblasts, and enhances the healing process of bone fracture. However, the underling mechanisms of EPO/EPOR signalling in skeletal development and regeneration remain unknown. Here, we aim to define the role of EPO/EPOR in chondrogenesis during skeletal development and regeneration. Our preliminary data show that EPO and EPOR are abundantly expressed in chondrocytes of prehypertrophic and hypertrophic zone of the growth plate while are weakly expressed in chondrocytes of the proliferating zone of the growth plate during different skeletal development stages of the mouse. The proliferation of primary chondrocytes is modestly increased under the treatment with mEPO indexed by BrdU incorporation assay. Alcian Blue staining for extracellular matrix proteoglycan indicates that mEPO promotes the differentiation of chondrocytes. This is accompanied by upregulated chondrogenic marker genes including Sox9, Sox5, Sox6, Collagen type 2, and aggrecan. Interestingly, we find that EPO and EPOR mRNA levels in chondrocytes are increased under hypoxia when compared to normoxic conditions, indicating EPO and EPOR might be mediators or targets of hypoxia regulated signals in chondrocytes. Our results suggest that EPO and EPOR are important mediators for chondrogenesis that deserves further investigation.

School of Biomedical Sciences

Cancer and Inflammation 2012 Symposium

5 June, 2012 (Tuesday)

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

Programme Summary

Symposium on Cancer & Inflammation 2012

5 June, 2012 (Tuesday)

Site Demonstration Lab (Room G02), Lo Kwee-Seong Integrated Biomedical Sciences Building

08:40-08:45 Opening Ceremony (Qi Min Zhan and Chi Hin Cho)

Time	*Topic*	*Speaker*	*Abstract No.*
08:45-10:10	**Cancer and Inflammation (1): Cell signaling and anti-cancer targets Chairpersons: Prof. Wing Tai Cheung and Prof. Ming Rong Wang**		
08:45-09:30	Plenary Lecture: The role of cell cycle protein Nlp in mitotic progression and tumorigenesis	Prof. Qi Min Zhan	PL-02
09:30-09:50	The host defense peptide LL-37 induces caspase-independent apoptosis via apoptosis-inducing factor and endonuclease G in colon cancer cells	Prof. Chi Hin Cho	S3-01
09:50-10:10	The involvement of S100A14 in cell invasion by affecting MMP-2 via p53-dependent transcriptional regulation	Prof. Zhi Hua Liu	S3-02

10:10-10:20 Photo taking & Tea Break

10:20-11:40	**Cancer and Inflammation (2): Cell signaling and anti-cancer targets Chairpersons: Prof. Michael S.C. Tam and Prof. Dong Xin Lin**		
10:20-10:40	A novel role for an orphan nuclear receptor ERRα in hypoxic growth regulation of prostate cancer	Prof. Franky L. Chan	S3-03
10:40-11:00	miR-106a is frequently upregulated in gastric cancer and inhibits the extrinsic apoptotic pathway by targeting FAS	Prof. Ning Zhi Xu	S3-04
11:00-11:20	Functional roles of FHL2 in liver cancers	Prof. Stephen K.W. Tsui	S3-05
11:20-11:40	Reciprocal activation between PLK1 and Stat3 contributes to survival and proliferation of esophageal cancer cells	Prof. Ming Rong Wang / Dr. Y. Zhang	S3-06

11:40-11:50 Tea Break

11:50-13:10	**Cancer and Inflammation (3): Anti-cancer targets and microRNAs in cancer Chairpersons: Prof. Alaster H.Y. Lau and Prof. Yan Ning Gao**		
11:50-12:10	Autocrine trypsin-PAR_2 signaling promotes cancer cell proliferation through miRNAs	Prof. Hong Ying Wang	S3-07
12:10-12:30	Small molecule activators of microRNA-34a with anti-cancer activities identified through library screening	Prof. Yang Chao Chen	S3-08
12:30-12:50	MicroRNAs and the metastasis of colorectal cancer	Prof. Jie Ma	S3-09
12:50-13:10	A xenograft-derived transcript dually encodes a CXC chemokine and a functional *Alu*-like non-coding RNA	Prof. Wing Tai Cheung	S3-10

13:10-13:25 Closing (Prof. Franky L. Chan)

PL-02

The role of cell cycle protein Nlp in mitotic progression and tumorigenesis

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Centrosome is a small cytoplasmic nonmembraneous organelles involved in the nucleation and organization of microtubules. It establishes polarity and orientation of microtubule during interphase, and contributes to assembly of spindle and chromosomal segregation. Therefore, centrosome aberrations may result in disruption of cell cycle progression, including chromosomal missegregation and aneuploidy, which often lead to cell transformation, tumorigenesis and the development of malignancies. The machinery that controls centrosome stability involves multiple important cellular proteins, including p53, BRCA1, Gadd45, p21, and Cdk2/cyclin E. The precise coordination among those regulators maintains centrosome duplication and stability. Here, we show that BRCA1 physically interacts and colocalizes with Nlp (Ninein like protein). Nlp centrosomal localization likely depends on normal cellular BRCA1 function since cells containing BRCA1 mutations or silenced for endogenous BRCA1 reveal disrupted Nlp colocalization to centrosomes. Interestingly, Nlp is modified by both Cdc2 and Aurora-B kinases. Suppression of endogenous Nlp results in aberrant spindle formation, failure of chromosomal segregation and cytokinesis, and aneuploidy. Nlp is overexpressed in human breast and lung carcinomas, and its deregulation is in part associated with *NLP* amplification. Nlp exhibits strong oncogenic property and induces NIH3T3 fibroblasts transformation. Importantly, Nlp transgenic mice mimic the phenotypes of disrupted BRCA1, including centrosome amplification and spontaneous tumorigenesis. Thus, Nlp may cooperatively act together with BRCA1 in mitotic machinery and abnormalities of Nlp leads to genomic instability and tumorigenesis.

The host defense peptide LL-37 induces caspase-independent apoptosis via apoptosis-inducing factor and endonuclease G in colon cancer cells

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Cathelicidin (LL-37), a human host defense peptide, plays pivotal roles in diverse biological processes, including the modulation of natural immunity, inflammation and tissue repair. However, the function of this peptide in tumorigenesis remains unclear. Our preliminary study showed that LL-37 was found to be significantly down regulated in human colon cancer tissues suggesting that this peptide could be a tumor suppressor gene in the development of colon cancer. In this study, the effect of LL-37 on human colon cancer cells was characterized. LL-37 induced extensive DNA fragmentation, chromatin condensation and phosphatidylserine externalization without causing caspase activation. Moreover, these effects were not blocked by caspase inhibitors. LL-37 induced apoptosis via downregulation of Bcl-2 and upregulation of Bak and Bax in a p53-dependent manner. In this connection, the pro-apoptotic effect of LL-37 was reversed by Bcl-2 overexpression, genetic ablation of Bax, or p53 siRNA. LL-37 also induced the upregulation and nuclear translocation of apoptosis-inducing factor (AIF) and endonuclease G (EndoG). Targeting on these genes by siRNAs rendered the cells resistant to LL-37-induced apoptosis. Above all, the pro-apoptotic effect of LL-37 was found to be mediated through a pertussis toxin-sensitive Gi-coupled receptor. Taken together, we demonstrated that LL-37 induced a caspase-independent apoptosis via the p53-Bcl-2/Bak/Bax and AIF/EndoG pathways. These findings open up a novel therapeutic avenue for the treatment of colon cancer using LL-37 as a therapeutic agent to induce apoptosis in cancer cells.

The involvement of S100A14 in cell invasion by affecting MMP-2 via p53-dependent transcriptional regulation

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S100 proteins have been implicated in tumorigenesis and metastasis. As a member of S100 proteins, the role of S100A14 in carcinogenesis has not been fully understood. Here, we show that ectopic overexpression of S100A14 promotes motility and invasiveness of esophageal squamous cell carcinoma (ESCC) cells. We investigate the underlying mechanisms and find that the expression of matrix metalloproteinase (MMP)-2 is obviously increased after S100A14 gene overexpression. Inhibition of MMP2 by a specific MMP2 inhibitor at least partly reverses the invasive phenotype of cells overexpressing S100A14. By serendipity, we find that S100A14 could affect p53 transactivity and stability. Thus, we further investigate whether the effect of MMP2 by S100A14 is dependent on p53. A series of biochemical assays show that S100A14 requires p53 to affect *MMP2* transcription and p53 potently transrepresses the expression of MMP2. Finally, RT-qPCR analysis of 52 human breast cancer specimens shows a significant correlation between *S100A14* mRNA expression and *MMP2* mRNA expression. Collectively, our data strongly suggest that S100A14 promotes cell motility and invasiveness by regulating the expression and function of MMP2 in a p53-dependent manner.

A novel role for an orphan nuclear receptor ERRα in hypoxic growth regulation of prostate cancer

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It is well known that adaptation of growth in a hypoxic tumor microenvironment is one of the major growth capacities and hallmarks of cancers, and this growth feature is being enabled by a number of growth signaling pathways, particularly induction of angiogenesis and reprogramming of energy metabolism to aerobic glycolysis in cancer cells. The effects of adaptive growth in hypoxia not only facilitate the survival of cancer cells in a microenvironment with reduced O_2 availability but also render cells resistance to various cancer therapies. The major adaptive response to hypoxia by cancer cells is the transcriptional regulation mediated by the hypoxia-inducible factor 1 (HIF-1). HIF-1 is a heterodimeric transcription factor consisting of HIF-1α subunit, its expression and transcriptional activity are tightly regulated by cellular O_2 level, and HIF-1β subunit, which is constitutively expressed. HIF-1 signaling is mostly controlled by protein levels of HIF-1α subunit, which undergoes continuous degradation in normoxia and is tightly regulated by a well-characterized O_2-dependent mechanism mediated by prolyl hydroxylase (PHD), von Hippel-Lindau (VHL)/Elongin-C/Elongin-B E3 ubiquitin ligase complex and proteasome. Recent advances also identify that HIF-1α stability is also regulated by an O_2-independent mechanism mediated by binding of heat-shock protein 90 (HSP90) and a multi-functional scaffold protein RACK1 to HIF-1α. In this lecture, Dr. Chan will discuss one novel mechanism on the regulation of HIF-1α stability by an orphan nuclear receptor ERRα (NR3B1) in prostate cancer cells, that has been recently characterized in his laboratory. Potential implications for this novel regulatory mechanism on prostate cancer development and treatment will be discussed.

miR-106a is frequently upregulated in gastric cancer and inhibits the extrinsic apoptotic pathway by targeting FAS

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Emerging evidence has shown the association of aberrantly expressed miR-106a with cancer development, however, little is known about its potential role in gastric carcinogenesis. In our present study, obviously overexpressed miR-106a was found in gastric cancer tissues compared with their non-tumor counterparts. Depression of miR-106a significantly inhibited gastric cancer cell proliferation and triggered apoptosis. Bioinformatic analysis combining with validation experiments identified FAS as a direct target of miR-106a. Rescue experiments and examination of caspase-8, PARP and caspase-3 further approved that miR-106a could inhibit gastric cancer cell apoptosis through interfering with FAS-mediated apoptotic pathway. Moreover, a significant inverse correlation was found between miR-106a and FAS expression not only in gastric cancer cell lines but also in gastric cancer specimens. Taken together, these findings suggest that ectopicly overexpressed miR-106a may play an oncogenic role in gastric carcinogenesis and impair extrinsic apoptotic pathway through targeting FAS.

Functional roles of FHL2 in liver cancers

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Four and a half LIM domain (FHL) proteins belong to a family of proteins that are able to mediate protein-protein interaction, bind to the cytoskeleton and trans-activate gene expression. Most recently, FHL1, FHL2 and FHL3, core members of the FHL protein family, were found to be tumour suppressors which are down-regulated in liver cancers. Our results show that FHL2 is downregulated in the majority of hepatocellular carcinoma (HCC) patients. Using stable overexpression clones of FHL2 in Hep3B cells, we show that FHL2 may mediate anti-proliferative effect through inhibition of the G1 to S cell cycle transition as evident from the decreased expressions of survivin and cyclin D1, as well as an increased expressions of cyclin-dependent kinase inhibitors p21 and p27. FHL2 overexpression also inhibits migration and invasion of HCC cells through epithelial-mesenchymal transition (EMT)-mediated pathway, as shown by reduction of mesenchymal marker vimentin and induction of epithelial marker E-cadherin. Surprisingly, we also demonstrated an anti-apoptotic function for FHL2 overexpression with increased resistance to doxorubicin-induced apoptosis, which indicates the separation of anti-proliferative and anti-apoptotic role of FHL2. To further elucidate the mechanism for aberrant transcription of FHL2, the potential promoter region was investigated. We found that the fragment from -138 to +292 in the promoter have positive regulatory effect. The bioinformatic analysis identified putative binding sites of five transcription factors (TFs). Of these TFs, Pax-5 and ZF5 expression was downregulated and correlated with the FHL2 expression in HCC tumor samples, indicating a possible role for these transcription factors in the regulation of FHL2 expression. Finally, we performed microarray analysis of the FHL2 knockout mice to identify new target genes of FHL2. Our data illustrate that FHL2 affects various genes involved in cell adhesion and motility, immune function, and transcription regulation. Of these genes, Lcn2, Bcl-6 and Creld2 belong to the category of cancer-related genes. We performed analysis of gene expression in clinical samples to identify genes relevant to human cancers and we identified the altered expression of Bcl-6 and Creld2 genes in HCC.

Reciprocal activation between PLK1 and Stat3 contributes to survival and proliferation of esophageal cancer cells

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Either aberrant Stat3 activation or PLK1 overexpression has been reported separately in various human cancers. However, the underlying mechanism and significance of Stat3 and PLK1 deregulation in carcinogenesis and cancer progression remain elusive. In the present study, we explored the potential relationship between Stat3 and PLK1 and the implications of their dysfunction in esophageal squamous cell carcinoma (ESCC). We demonstrated that Stat3 directly activated transcription of PLK1 in esophageal cancer cells and NIH3T3. PLK1 then potentiated the expression of Stat3; β-catenin was involved in PLK1-dependent transcriptional activation of Stat3. This mutual activation between Stat3 and PLK1 was required for proliferation of esophageal cancer cells and resistance to apoptosis in culture and as tumor xenografts in mice. Furthermore, phosphorylation of Stat3 and overexpression of PLK1 were correlated in a subset of ESCC. Our data indicate that Stat3 and PLK1 control each other's transcription in a positive feedback loop that contributes to the development of ESCC. Increased activity of Stat3 and overexpression of PLK1 promote survival and proliferation of ESCC cells in culture and in mice. Stat3 and PLK1 might be targeted for the treatment of ESCC harboring hyperactivated Stat3 and PLK1.

Autocrine trypsin-PAR_2 signaling promotes cancer cell proliferation through miRNAs

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Tumor microenvironment is replete with serine proteinases. As a sensor of proteinases, proteinase activated receptors (PARs) play critical roles in tumorigenesis. PAR_2 is the only member in the family which can be activated by trypsin. We showed that several different colon cancer cell lines express trypsin and PAR_2 at the same time. Knockdown of PAR_2 significantly reduced cell proliferation, which shown by BrdU labeling assay. In the cell which expressed PAR_2 but not trypsin, activation of PAR_2 with activating peptide stimulated DNA synthesis. Further studies showed that activation of PAR_2 stimulated cell proliferation through cyclin D1-E2F pathway. More importantly, couples of microRNAs were changed followed by autocrine PAR_2 signaling, such as miR-15a and miR-16. Supplement of miR-15a blocked autocrine-induced cell proliferation. Moreover, knockdown of PAR_2 in HT-29 cells reduced tumorigenicity in nude mice. Our findings provide the first evidence that autocrine proteinases signaling promotes cancer cell proliferation through miRNAs.

Small molecule activators of microRNA-34a with anti-cancer activities identified through library screening

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MicroRNAs play critical roles in various biological processes through regulating gene expression. Aberrant expression of microRNAs has been well documented in a variety of cancers. microRNAs function as oncogenes or tumor suppressors and represent promising therapeutic targets for cancer treatment. Small molecules modulating microRNA expression would thus constitute potential anti-cancer agents. microRNA-34a functions as a tumor suppressor and is downregulated or silenced in various cancers including hepatocellular carcinoma (HCC). In this study, we established the microRNA-34a luciferase report system and developed an assay for screening small molecule activators of microRNA-34a. The natural product library (Timtec) containing 640 pure compounds was screened to identify microRNA-34a activators. Two compounds were identified to be microRNA-34a activators. These two compounds dramatically activated microRNA-34a expression in HCC cells with microRNA-34a silencing. It was further demonstrated that these two compounds exhibited growth inhibiting activities on various HCC cell lines but not in non-tumorigenic human hepatocytes. These two compounds also downregulated the expression of microRNA-34a target proteins such as cyclin D1. The in vivo anti-cancer efficacy of these two compounds is under investigation.

MicroRNAs and the metastasis of colorectal cancer

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Metastasis is the main cause of mortality in patients with solid tumours. Recently, microRNAs (miRNAs) have been discovered to have a role in metastasis. In the present study, we investigated the miRNA expression profiles of 8 primary CRC (colorectal cancer) patients with or without lymph node metastasis using an miRNA microarray and identified that miR-145 displayed dramatic upregulation in CRC with lymph node metastasis. The precise expression of miR-145 was determined using qRT-PCR in 120 primary CRC patients with or without lymph node metastasis. The miR-145 showed dramatically higher expression in CRC with lymph node metastasis than without lymph node metastasis. Whether the upregulation of miR-145 affects colon cancer metastasis, we performed miR-145 gain-of-function studies in human CRC cells. Our results showed that miR-145 up-regulation could promote colon cancer cell migration and invasion in vitro and in vivo. In addition, we sought to identify cellular proteins which were directly or indirectly regulated by miR-145 using iTRAQ labeling and 2DLC-ESI-MS/MS. Our analyses indicated that HSP27 was up-regulated in miR-145 over-expressed CRC cells. We also found that knockdown of HSP-27gene expression by siRNA reversed miR-145-mediated induce of HCT-8 cell migration. Collectively, these findings suggested that mir-145 might play an important role in metastasis of CRC.

A xenograft-derived transcript dually encodes a CXC chemokine and a functional *Alu*-like non-coding RNA

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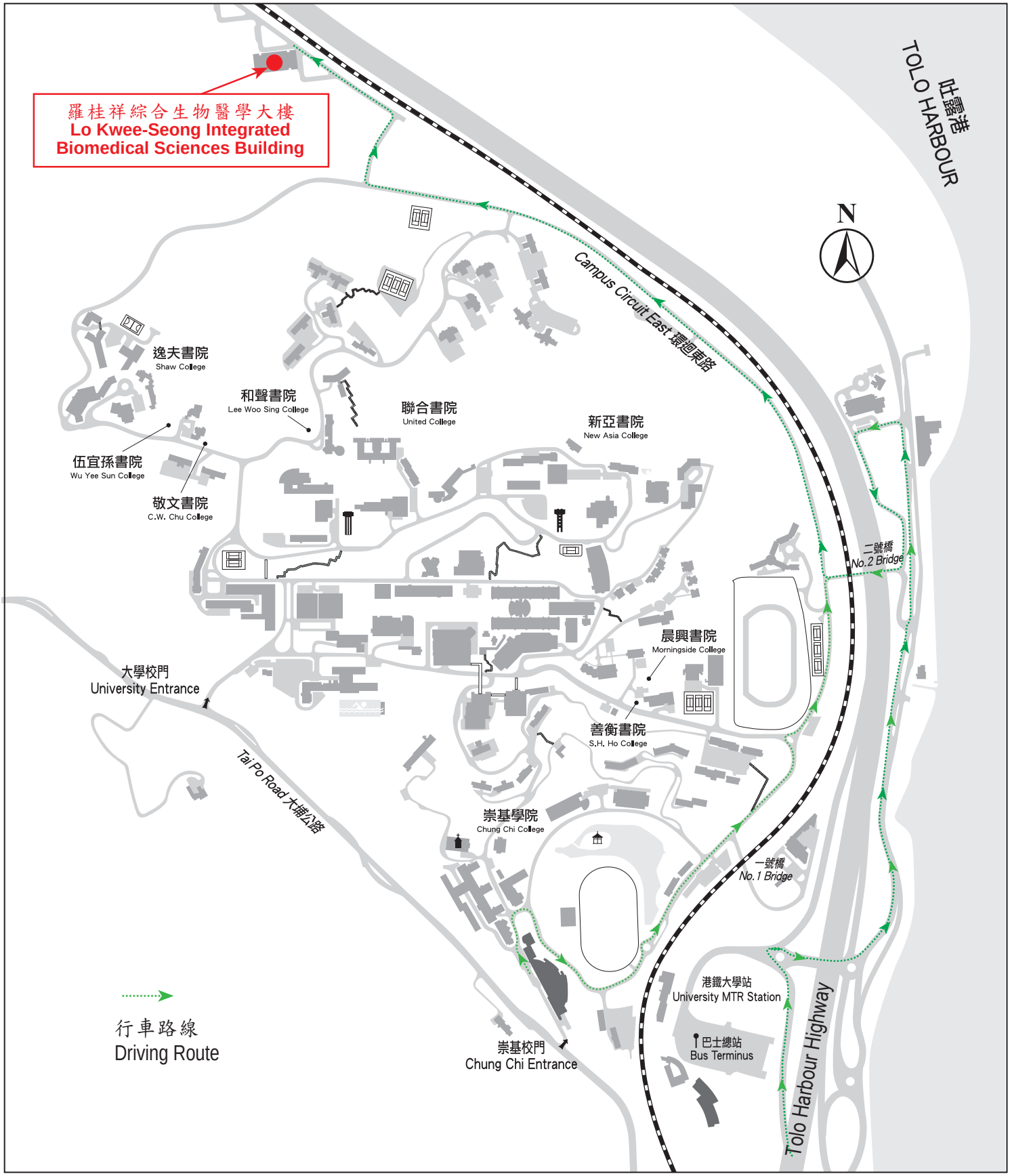
A novel 1199b-transcript that was originally isolated from xenograft induced by GPCR mas-overexpressing cells. In the 5'-end of the transcript, an ORF was found to encode a 101-amino acid protein. Sequence analysis suggested the encoded protein is a novel CXC chemokine named as Tumour-induced Factor (TIF) which was predicted to be a member of the GRO family. Indeed, functional studies indicated that TIF potently induced neutrophil migration and angiogenesis. Unexpectedly, cells overexpressing TIF suppressed xenograft formation when co-injected with mouse embryonic fibroblasts.

The TIF transcript was also characterized with a long 3'-untranslated region containing a stretch of 71 nt (TIFm71) that was predicted to fold into a stem-loop structure. Intriguingly, TIFm71 was found to share sequence homology with pre-miRNAs and rodent repetitive *Alu*-like element. Results of studies indicated that TIFm71 was processed out from TIF transcript and could function as a noncoding regulatory RNA mediating epithelial-mesenchymal transition. Consistently, cells overexpressing TIFm71 were found to show a higher cellular mobility in a wound healing assay and an increased invasion ability in a Matrigel assay.

Alu element is the most abundant repetitive sequence in human genome and its function remains elusive. The notion that *Alu*-like sequence functions as a short noncoding regulatory RNA which mediates epithelial-mesenchymal transition, would shed a new light on the ways how *Alu* element modulates cellular functions. Importantly, upon proper processing of such dually encoded mRNAs would activate a network rather than a single signalling pathway.

Direction to Lo Kwee-Seong Integrated Biomedical Sciences Building



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