The Chinese University of Hong Kong

Faculty of Medicine

School of Biomedical Sciences

Postgraduate Research Day 2012

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Welcome Message from the Director of School of Biomedical Sciences

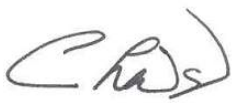
I am most delighted to welcome you all to the *SBS Postgraduate Research Day 2012*, the annual flagship event of the School of Biomedical Sciences organized solely by and for our students. Stepping into its third year, the *Postgraduate Research Day 2012* continues to serve as an important platform for our students to showcase their talents and achievements and to interact with their peers and supervisors.

Different from before, the *SBS Postgraduate Research Day 2012* will be held in our new home, the Lo Kwee-Seong Integrated Biomedical Sciences Building, Northside Research Campus. It is our hope that the openness and cohesiveness of this fit-for-purpose design new home will create an ideal environment which favors free scientific exchange and vigorous scholarly interactions between our students and investigators. I am sure *Postgraduate Research Day 2012* held in this new locale will be more successful than the previous ones.

It is the vision of our School to nurture future scientists who are abreast of biomedical advances and are able to do cutting-edge research. This cannot be achieved single-handedly by our investigators − a young, creative and dynamic student body thirsting for limitless knowledge and intellectual advancement is of equal importance in materializing our vision. Committing oneself to research is a long, painstaking and winding journey full of frustrations and surprises. As the proverb goes, "Rome was not built overnight" −persistence and patience with curiosity, prudence, courage as well as ethical mind are all the indispensible qualities for becoming a successful and respected researcher. Besides facilitating our students to achieve research and academic excellence, it is our goal to provide holistic education so that our students can excel in whichever career they choose to pursue after they graduate. I believe the annual *Postgraduate Research Day* offers the best opportunity for our students to sharpen their other attributes such as leadership, flexibility, devotion, bondage, and mutual trust.

I would like to take this opportunity to thank all individuals involved in planning, organizing, and coordinating this event, particularly members of the Organizing Committee of *SBS Postgraduate Research Day 2012* for their hard-work, commitment and time that make the event a reality. I would also like to extend my earnest gratitude to the Graduate Education Office for its continued support to our graduate education and students.

On behalf of all staff of the School of Biomedical Sciences, I wish you all a successful *Postgraduate Research Day 2012*.



Wai-Yee Chan. Ph.D.
Professor of Biomedical Sciences &
Director, School of Biomedical Sciences
November 2012

School of Biomedical Sciences

Postgraduate Research Day 2012

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Program of SBS Postgraduate Research Day

15th Nov, 2012 (Thursday)

Time	Event	Venue
9:00 - 9:15 am	Registration	G/F LKS
9:15 - 9:25 am	Opening Speech	G02 LKS
9:25 - 9:30 am	Photo Session	G02 LKS
9:30 - 11:00 am	Poster Presentation (Reproduction, Development and Endocrinology)	G/F LKS
11:00 am - 12:20 pm	Poster Presentation (Vascular and Metabolic Biology)	G/F LKS
-Break Time-		
1:30 - 3:20 pm	Poster Presentation (Cancer and Inflammation)	G/F LKS
3:20 - 4:00 pm	Poster Presentation (Neuro-degeneration, development and Repair)	G/F LKS
4:00 - 4:30 pm	Poster Presentation (Stem Cell and Regeneration)	G/F LKS

*LKS — Lo Kwee-Seong Integrated Biomedical Sciences Building

16th Nov, 2012 (Friday)

Time	Event	Venue
9:00 - 9:15 am	Registration	G02 LKS
9:15 - 10:30 am	1st Oral Presentations	G02 LKS
-Tea Break-		
10:45 - 12:00 am	2nd Oral Presentations	G02 LKS
-Tea Break-		
12:15 - 12:45 pm	Prize-giving Ceremony	G02 LKS

*LKS — Lo Kwee-Seong Integrated Biomedical Sciences Building

Cancer and Inflammation

Title of Poster Presentation	Name	Abstract No.
A novel role of CRMP1 in the epithelial-mesenchymal transition regulation in prostate cancer	G.H. Cai	C1
Deterioration of mouse femur microstructure by heavy cigarette smoking and the effect of smoking cessation	R. L.Y. Chan	C2
Purification and characterization of a hemagglutinin from the Northeast China Black Bean cultivar of Phaseolus vulgaris	X.L. Dan	C3
Reversal of P-glycoprotein mediated multidrug resistance by Cryptotanshinone and Dihydrotanshinone	T. Hu	C4
Identification of Putative Targets of Tumor Suppressor miR-218 in Human Pancreatic Ductal Adenocarcinoma	C.H. Li	C5
The biochemical characteristics of a exonic small Non coding RNA, TIFm71	L, Zhang	C6
Orphan nuclear receptor TLX functions to promote hormone-resistant growth of prostate cancer cells via its suppression of androgen receptor gene expression	L. Jia	C7
TCP-1 as a novel phage-display peptide targeting colon cancer	L. Lu	C8
PPAPs-induced p21 upregulation: a selective mechanism versus cancer	K.K. Miu	C9
Osteopontin and Mast Cell	C.W. Ng	C10
Marmorin, a mushroom type I ribosome inactivating protein (RIP), halts the growth of breast cancer cells in vitro and in vivo	W. L. Pan	C11
Blood biomarker of pyrrolizidine alkaloids and their N-oxides associated hepatotoxicity	J.Q. Ruan	C12

miR-490-3p is dysregulated in Helicobacter pylori associated gastric cancer and can inhibit cancer cell growth through autophagy	J. Shen	C13
Dihydrotanshinone induced apoptosis in colon cancer cells by p53-independent but ROS dependent pathway	L. Wang	C14
Proteomic research on Hpn-induced HCC apoptosis	W.M. Wang	C15
Regulatory role of an orphan nuclear receptor LRH-1 in castration resistant prostate cancer	L.J. Xiao	C16
Metabolic conversion of pyrrolizidine alkaloid N-oxides to pyrrolizidine alkaloids in both liver and intestine	M.B. Yang	C17
Inhibitory effects of lactoferrin derivatives on Candida albicans which causes infections in diabetics	C.M. Yin	C18
Cathelicidin is a host defense peptide in controlling Helicobacter pylori survival and infection	L. Zhang	C19
Small molecule activators of microRNA-34a with anti-cancer activities identified through library screening	Z.G. Xiao	C20

Title(C1): A novel role of CRMP1 in the epithelial-mesenchymal transition regulation in prostate cancer

G.H. Cai, S. Yu, Y.P. Ji and F.L. Chan

Program: PhD; Supervisor: CHAN Leung Franky

Collapsin response mediator protein 1 (CRMP1) is originally identified as a cytoplasmic phosphoprotein involved in the semaphorin 3A (Sema3A)-induced growth cone collapse during neural development, via a mechanism of regulation of cytoskeleton dynamics. Epithelial-to-mesenchymal transition (EMT) refers to a phenomenon that the well-polarized epithelial cell transforms to non-polarized fibroblast-like cells with cytoskeleton reorganization. This process, which facilitates cell motility and migration, occurs during normal development. Besides, EMT also occurs in caner development, particularly during metastasis. As CRMP1 is a cytoskeleton regulator, we hypothesize that CRMP1 may be involved in the EMT in prostate cancer development.

Our preliminary expression study showed that CRMP1 exhibited a reduced expression pattern in high Gleason-scored clinical prostate cancer tissues and also in prostatic epithelial cells (BPH1) and prostate cancer cells (DU145) with overexpression of EMT-inducing transcription suppressor Snail and Slug. We found that knockdown of CRMP1 induced EMT in human prostate cancer cell DU145, and ectopic expression of CRMP1 initiated mesenchymal to epithelial transition (MET) in DU145 and another human prostate cancer cell PC3, which were evidenced by the change of cell morphology and the expression levels of epithelial and mesenchymal markers. Phenotype studies revealed that CRMP1 inhibited cell proliferation, migration and invasion of DU145 and PC3 cells. In vivo study showed that CRMP1 suppressed the growth of DU145 tumor xenograft. Results of CRMP1 promoter-driven luciferase reporter assay and ChIP assay further indicated that Snail was a direct transrepressor of CRMP1 and might be responsible for the loss of CRMP1 expression in EMT.

Taken together, our findings suggest that CRMP1 might promote MET and suppress the development of prostate cancer.

Title(C2): Deterioration of mouse femur microstructure by heavy cigarette smoking and the effect of smoking cessation

R.L.Y. Chan and C.H. Cho

Program: PhD; Supervisor: CHO Chi Hin

Although cigarette smoking is considered as a risk factor of osteoporosis, the underlying mechanism is still unknown. To unveil the mechanism and investigate the effect of smoking cessation, a mouse smoking model was used. For determining the dose effect of smoking, young BALB/c mice were subjected to atmospheric air, 2% (v/v) or 4% (v/v) cigarette smoke exposure in a smoke chamber for 1 hour per day, 6 days per week for a total of 14 weeks. For evaluating the effect of smoking cessation, mice were given with atmospheric air or 4% cigarette smoke for 14 weeks. The smoking groups were either continuing to smoke or stopping smoking for a further period of 6 weeks. Control animals continued to expose to atmospheric air for a total of 20 weeks. Mineral and collagen loss in urine and serum bone turnover marker was measured during the experimental period. At the conclusion of the experiment femora and tibias were harvested for the assessment of mineral content and histology of femur. Bone microstructure was also analyzed by micro-CT scanning.

Results from the first set of experiment showed that deoxypridinoline (DPD), calcium and phosphorus excretion in urine increased in the 4% smoke group. Serum alkaline phosphatase (ALP) activity increased significantly in the 4% smoke group. It was found that the dry weight, ash weight, calcium and phosphorus contents of femora and tibias had no significant difference in all groups. The relative bone volume (BV/TV) and trabecular thickness (Tb.Th) were significantly lower in the 4% smoke group but without affecting trabecular number (Tb.N) and trabecular spacing (Tb.Sp) in the micro-CT scanning. The structure model index (SMI) of 4% smoke group was significantly elevated, suggesting a weaker bone microstructure. After 6 weeks of smoking cessation, the body weight of smoking mice returned to control level but the bone weight and bone mineral content had no significant change. The serum ALP level decreased significantly after smoking cessation. Urine DPD level in smoking groups were still significantly higher than the control group. The trabecular structure of distal femur was shown less intact in smoking groups, in addition to the reduced thickness in femur growth plate.

In conclusion, chronic heavy smoking increases bone turnover and deteriorates bone microstructure in mice while moderate smoking does not. Smoking cessation for 6 weeks does not reverse the ill effects of chronic heavy smoking on bone.

Title(C3): Purification and characterization of a hemagglutinin from the Northeast China Black Bean cultivar of Phaseolus vulgaris

X.L. Dan, J.H. Wong and T.B. Ng

Program: PhD; Supervisor: NG Tzi Bun

A 66-kDa hemagglutinin was isolated from the Northeast China Black Bean by using affinity chromatography on Affi-gel blue gel, ion exchange chromatography on Mono Q followed by gel filtration on Superdex 75 with a FPLC system. The hemagglutinin was found to be stable in pH ranging from 2 to 11 and temperature ranging from 20℃ to 60℃. The hemagglutinin activity could be inhibited by divalet cation (Cu2+, Mg2+, Mn2+, Ca2+, Zn2+, Fe2+) and a trivalent cation (Fe3+). However, its hemagglutinating activity could not be inhibited by a variety of sample sugar tested. The hemagglutinin exhibited potent anti-proliferation activity on MG63 human osteosarcoma cells with an IC50 of 5 μM. Apoptosis of hemagglutinin treated MG63 cells was observed by chromatin staining with Hoechst 33342. Tumor cell migration activity was greatly reduced after treatment with this hemagglutinin using transwell assay and the effect was discernible even at low hemagglutinin dosage. The results reveal the potential of this hemagglutinin in the treatment of osteocarcinoma.

Title(C4): Reversal of P-glycoprotein mediated multidrug resistance by Cryptotanshinone and Dihydrotanshinone

T. Hu, J.H.K. Yeung, L. Wang, X.L. Zhou, P.M.Y. Or, K.K.W. To and C. H. Cho

Program**:** PhD; Supervisor: CHO Chi Hin

Multidrug resistance of cancer cells is an obstacle to successful cancer chemotherapy. Overexpression of P-glycoprotein (P-gp), an ATP-binding cassette (ABC) membrane transporter, can mediate the efflux of drugs out of cancer cells, leading to multidrug resistance and chemotherapy failure. Therefore, development of safe and effective P-gp inhibitors plays an important role in chemotherapy.

This study investigated the reversal of P-gp mediated multidrug resistance by several tanshinones including Tanshinone I, Tanshinone IIA, Cryptotanshinone and Dihydrotanshinone isolated from Danshen (*Salvia miltiorrhiza*), a traditional Chinese medicine. Bi-directional transport assay in Caco-2 cells showed that Cryptotanshinone and Dihydrotanshinone could decrease digoxin efflux ratio in a concentration-dependent manner, indicating inhibitory effects on P-gp function. Whereas, Tanshinone I and Tanshinone IIA had no effects. Moreover, Cryptotanshinone and Dihydrotanshinone could increase the intracellular accumulation of doxorubicin in P-gp overexpressing SW620 Ad300 cancer cells. To this end using MTT assay Cryptotanshinone and Dihydrotanshinone could potentiate the cytotoxicity of doxorubicin and irinotecan in SW620 Ad300 cells as well. These findings suggest that Cryptotanshinone and Dihydrotanshinone could be used as an adjuvant therapy together with anticancer drugs in chemotherapy to improve their therapeutic effects for colon cancer.

Title(C5): Identification of Putative Targets of Tumor Suppressor miR-218 in Human Pancreatic Ductal Adenocarcinoma

C.H. Li, Y.C. Chen

Program: PhD; Supervisor: CHEN Yangchao

In pancreatic ductal adenocarcinoma (PDAC), microRNAs dysregulation is frequently observed and linked to malignant development (1-3). miR-218 was frequently reduced in pancreatic cancers and PDAC cell lines, but its role involved is not deeply investigated. To delineate tumorigenic events occured after loss of miR-218, identification and characterization of putative miR-218 targets with critical functions are essential to reveal the cancer-related network.

Intratumoral injection of miR-218 mimics inhibited subcutaneous tumor growth which demonstrated the tumor suppressing effect of miR-218 in PDAC. By candidate gene approach, we selected genes that were reported targets of miR-218, or were predicted by bioinformatics tools. Among them, the mRNA levels of ROBO1, VOPP1 and UGT8 were reduced after miR-218 mimics transfection. ROBO1 and VOPP1 are reported as a putative miR-218 targets that possesses various protumorigenic effects (4-5), whereas the roles of UGT8 were yet to be examined. 3'-UTR reporter assay showed transfection of miR-218 mimics inhibited the activity of luciferase reporter containing the 3'-UTR of UGT8. UGT8 protein level was reduced after overexpression of miR-218 in SW1990 which showed that miR-218 negatively regulated the functional UGT8 gene product. Profiling of UGT8 transcript and protein level in normal human pancreatic ductal epithelial cells and several PDAC cell lines showed that UGT8 level was positively correlated with the metastatic potential of PDAC cells. Taken together, UGT8 was a promoting factor during cancer progression, and loss of miR-218 in PDAC was the underlying reason for the aberrant upregulation of UGT8.

We demonstrated that miR-218 has potential tumor suppressor roles in PDAC by regulating several pro-tumorigenic factors. Loss of miR-218 in PDAC leaded to the increase of ROBO1 and VOPP1 that contributed to cell invasion and cell proliferation respectively. Most importantly, we identified a novel target UGT8 and that its upregulation in PDAC was associated with PDAC metastasis.

Title(C6): The biochemical characteristics of a exonic small Non coding RNA, TIFm71

L., Zhang

Program: PhD; Supervisor: CHEUNG Wing Tai

Noncoding RNAs which do not encode proteins, are involved in many biological processes and are increasingly seen important. The transcript of a novel CXC chemokine TIF(Tumor-Induced Factor) which identified originally from mass-overexpressed cell induced-xenograft has a long 3'-UTR containing a stretch of 71 nt (TIFm71). Our study has shown that this fragment can be processed out from 3'-UTR of TIF mRNA and can play regulatory role independently. TIFm71 was predicted to fold into a stem loop structure which is similar to pre-miRNAs and share a high sequence homology with rodent Alu-like element. Using TIFm71 overexpression stable cell line, we found that TIFm71 can mediate epithelial-mesenchymal transition (EMT) and show higher cell mobility through wound healing assay. MTT assay shows that TIFm71 can suppress cell proliferation. Western Blot shows a higher phosphorylated ERK protein level which may indicate that TIFm71 can play its function through ERK pathway.

The Alu elements are conserved repeat sequences that belong to the SINE family of retrotransposons found abundantly in primate genomes and its function remains elusive up to date. In order to check whether Alu element has a similar function as TIFm71, constructs containing Alu elements were transfected into HEK293 cells and MTT assay also show an inhibition of cell proliferation. Interestingly, western blot also showed a upregulated phosphorylated ERK in Alu element overexpressed cells which consistent with TIFm71. Alu-like element function as non coding RNA may provide a new direction to explore functions of Alu element.

Title(C7): Orphan nuclear receptor TLX functions to promote hormone-resistant growth of prostate cancer cells via its suppression of androgen receptor gene expression

L. Jia, D.L. Wu, S. Yu and F.L. Chan

Program: PhD; Supervisor: CHAN Leung Franky

TLX is an orphan nuclear receptor that is characterized to play an important regulatory role in embryonic and adult neural stem cells on their self-renewal. Other than the only observation so far that its overexpression in nervous tissues may contribute to the initiation and development of brain tumors, the significance of TLX in malignancies is still totally unclear.

In a preliminary expression profile study we observed that TLX exhibited increased expression patterns in three in vitro models of androgen-independent and antiandrogen-resistant prostate cancer, and also in high-grade and hormone-refractory clinical prostate cancers. To elucidate its regulatory role in prostate cancer cell growth, we generated stable TLX-overexpressed infectants in two prostate cancer cell lines (LNCaP, VCaP) with low endogenous TLX levels for their growth characterization studies. Intriguingly, overexpression of TLX in LNCaP facilitated both androgen-independent and antiandrogen-resistant cell growth features in vitro, and promoted tumor growth capacities in castrated male SCID mice, while LNCaP-shTLX cells with stable TLX-knockdown could enhance their sensitivity to antiandrogen as compared with parental cells. Expression studies showed that both LNCaP-TLX and VCaP-TLX cells exhibited decreased expressions of androgen receptor (AR) and AR-target genes. Results of luciferase-based reporter assay also revealed that overexpression of TLX in prostate cancer cells LNCaP and non-prostatic cells HEK293 could suppress the AR transcriptional activity in the presence or absence of androgen (DHT). Chromatin immunoprecipitation analyses on HEK293 demonstrated the direct transrepressive regulation of TLX on AR gene promoter, while histone demethylase LSD1 might act as a corespressor of TLX since knockdown of LSD1 leaded to de-repression of AR target genes in LNCaP-TLX cells.

Based on the preliminary results, it is hypothesized that overexpression of TLX might contribute to the hormone-resistant prostate cancer cell growth and advanced progression of prostate cancer.

Title(C8): TCP-1 as a novel phage-display peptide targeting colon cancer

L. Lu and C.H. Cho

Program: PhD; Supervisor: CHO Chi Hin

TCP1 is a novel vasculature-targeting peptide. It was discovered through the *in vivo* phage library selection. It was demonstrated that TCP1 peptide exhibited the homing ability to the tumor neovasculature and was capable of efficiently delivering imaging agents and chemotherapeutic drugs to this target site. The current study is to further investigate the targeting ability of TCP1 to deliver anti-cancer drugs and imaging agents, and to demonstrate the specificity of TCP1 on the putative target on tumor blood vessels and explore the potential mechanisms.

In this study we injected GFP or TCP-1/GFP into colon tumor-bearing mice. After 2-hour circulation, we observed from the frozen sections that TCP-1 peptide delivered GFP only to tumor blood vessel other than normal organs. To demonstrate TCP1 has the ability for targeted delivery of TNF-α, we injected TNF-α or TCP-1/TNF-α into tumor-bearing mice. They were killed and various organs were collected after 24 h. Frozen sections were prepared. Numbers of apoptotic cells and microvessels in colon tumors were assessed as therapeutic activity through microvessel density and TUNEL assays. Results showed that TCP-1/TNF displayed stronger effects than TNF-α alone in the induction of apoptosis and reduction in number of microvessels in the tumors without significant effect in systemic toxicity.

To identify the cell surface targets for TCP1, we incubated the colon 26 cells with biotin-TCP1 probe peptide and performed the pull-down assay using the strong biotin-avidin interaction by neutravidin-agarose beads. Preliminary western-blot data showed that some membrane proteins on colon 26 cells could bind to TCP1 after incubation with biotin-TCP1 probe.

Taken together, TCP1 peptide appears to be a promising agent in molecular imaging and drug delivery for gastrointestinal cancers. Our peptide was also found to be able to bind to tumor blood vessels and some colorectal cancer cells. The potential mechanisms will be further clarified in future study.

Title(C9): PPAPs-induced p21 upregulation: a selective mechanism versus cancer?

K.K. Miu, K.W. To and G. Lin

Program: MPhil/PhD; Supervisor: LIN Ge

A novel class of potential anti-cancer compounds—Polycyclic Polyprenylated Acylphloroglucinols (PPAPs) was recently isolated from *Garcinia* species. These compounds have cytotoxicity selective towards human colon cancer cells to normal cells at similar concentrations. Among them, Guttiferone K (GutK) and Oblongifolin C (OblongC) were investigated and reported to promote cell cycle arrest through multiple mechanisms, including induction of cell cycle inhibitor p21. However, it is now known that similar cancer cytotoxicity is not found in liver cell types.

The experiments are set to investigate whether PPAPs-induced p21 upregulation can be a potential anti-cancer mechanism for PPAPs to serve as future drugs. Perform cytotoxicity screens for liver cancer cell susceptibility to various PPAPs and dose-dependent toxicity of GutK & OblongC to colon cancer cell lines. Perform Western blotting to confirm p21 protein induction from cells treated with GutK and perform RT-PCR to monitor gene expression levels of p21. Liver cancers are resistant towards PPAPs at similar concentrations otherwise effective in colon cancer. p21 is confirmed to be upregulated and induce cell cycle arrest. The underlying induction mechanism is under ongoing research.

Investigation into PPAPs-induced p21 upregulation will provide more clues to the mechanism for their attractive anti-cancer property. It is hopeful that we can identify a safe dose for PPAPs that is toxic to cancer yet leaving normal tissues unharmed. Subsequently, the project shall extend to investigate the gene expression levels of related transcription factors to understand the rationale for p21 protein induction.

Title(C10): Osteopontin and Mast Cell

C.W. Ng, Y.S. Tam and H.Y.A. Lau

Program: PhD; Supervisor: LAU Hang Yung Alaster

The concentration of the multifunctional matrix glycoprotein, osteopontin (OPN) is increased in inflammatory tissue and is believed to modulate the functions of inflammatory cells. Recently, OPN has also been found to be involved in regulating inflammatory responses in mast cell. In the current study, we investigated the effects of OPN on inflammatory responses (Histamine release, IL-8 synthesis, adhesion and chemotaxis were determined by spectrofluorometric, ELISA assay, fluorescent assay and transwell assay respectively) of human mast cells. OPN extracted from human milk and human mast cells cultured from $CD34^{+}$ monocytes isolated from peripheral blood were employed. We found that suspended human milk OPN but not recombinant OPN was able to inhibit anti-IgE induced histamine release only in the presence of manganese. OPN could also suppress the chemotaxis of immature mast cells induced by CCL11. When OPN was coated to culture plates, it was found to mediate adhesion of mast cells. Upon adhesion of the mast cells to OPN, anti-IgE induced IL-8 synthesis was reduced dose dependently but not histamine release. These studies suggest that human milk extracted OPN may be able to alleviate the inflammatory responses of human mast cell toward antigen, therefore affecting pathological conditions related to mast cell.

Title(C11): Marmorin, a mushroom type I ribosome inactivating protein (RIP), halts the growth of breast cancer cells in vitro and in vivo

W. L. Pan, J. H. Wong and T. B. Ng

Program: PhD; Supervisor: NG Tzi Bun

Breast cancer is the second most common cancer. In this study, the mechanism of antitumor activity of marmorin, a type I RIP from the mushroom Hypsizigus marmoreus, on breast cancer was investigated. Marmorin evinced more potent cytotoxicity toward estrogen receptor (ER)-positive MCF7 breast cancer cells (IC50 = 5 μM) than ER-negative MDA-MB-231 cells (IC50 = 15 μM) but has no inhibitory effect on normal nasopharyngeal epithelial NP69 cells. Interestingly, marmorin inhibited dose-dependent proliferation of human umbilical vein endothelial cells which is critical to angiogenesis. Marmorin did not affect the the expression level of estrogen receptor β (ERβ), but reduced that of estrogen receptor α (ERα) which has been demonstrated to counter both cell cycle checkpoint and anti-apoptotic protein Bcl-2. 17β-estradiol induced proliferation of MCF7 cells was significantly inhibited by marmorin, suggesting that the ER-mediated pathway was involved in inhibition of marmorin on ER-positive breast cancer cells. Moreover, marmorin induced time-dependent apoptosis in both MCF7 and MDA-MB-231 cells. Marmorin dramatically caused G2/M-phase arrest and mitochondrial membrane potential depolarization in MCF7 cells, and to a lesser extent in MDA-MB-231 cells. Finally, in an in vivo xenograft tumor model involving MDA-MB-231 cells, intraperitoneal injection of marmorin (2 mg/kg/d) significantly reduced the tumor volume and weight compared with vehicle control. In summary, the RIP marmorin exhibited higher inhibitory potency in ER-positive MCF7 breast cancer cells than ER-negative MDA-MB-231 cells partially due to ERα-mediated mitochondrial signaling pathway. The results suggest that marmorin is a potential candidate for breast caner therapy.

Title(C12): Blood biomarker of pyrrolizidine alkaloids and their N-oxides associated hepatotoxicity

J.Q. Ruan, G. Li

Program: PhD; Supervisor: LIN Ge

Intake of pyrrolizidine alkaloids (PAs) and/or PA N-oxides present in plants and/or contaminated in foodstuffs causes numerous cases of hepatotoxicity, especially hepatic sinusoidal obstruction syndrome (HSOS) worldwide, however, to date, no method is available for specifically assessing PA/PA *N*-oxide intoxication. We proposed pyrrole-protein adducts derived from the metabolic activation of PAs/PA *N*-oxides as a potential biomarker for diagnosing PA/PA *N*-oxide-induced hepatotoxicity, and successfully determined blood pyrrole-protein adducts in HSOS patients resulting from the intake of PA/PA *N*-oxide-containing herbs.

Blood pyrrole-protein adducts in HSOS patients were quantified using our newly developed UHPLC-TQ-MS method. Further confirmation was conducted in PA/PA *N*-oxide-treated mice. Hepatotoxicity in mice was evidenced by liver morphological changes, serum ALT level elevation, and endothelial cell damage determined by immunohistochemistry assay. Blood pyrrole-protein adducts were detected in all ten HSOS patients, but not in patients with other liver diseases. Blood pyrrole-protein adducts levels appeared proportional to hepatotoxic severity in both HSOS patients and the treated mice. Moreover, seneciphylline *N*-oxide and senecionine *N*-oxide were found as predominant ingredients in all available herbs ingested by HSOS patients, demonstrated that all HSOS patients examined were caused by PA *N*-oxides.

PA *N*-oxides were proven to cause HSOS in human for the first time. Blood pyrrole-protein adducts in HSOS patients was quantified by a novel UHPLC-TQ-MS method and revealed to be proportional to hepatotoxic severity, thus these adducts could serve as a biomarker of PA/PA *N*-oxide-induced hepatotoxicity including HSOS.

Title(C13): miR-490-3p is dysregulated in Helicobacter pylori associated gastric cancer and can inhibit cancer cell growth through autophagy

J. Shen and C.H. Cho

Program: PhD; Supervisor: CHO Chi Hin

MicroRNAs (miRNAs) are small, non-coding RNAs that regulate gene expression mainly by binding to the 3' untranslated regions (3'-UTRs) of target genes. They are important post-transcriptional regulators and their dysregulation is seen in a wide range of pathological conditions including carcinogenesis. In the present study, using miRNA array we found a gradual down-regulation of miR-490-3p in our animal model in the development of *Helicobacter pylori*- associated gastric cancer deriving from inflammation and intestinal metaplasia (IM). This down-regulation was further validated in human normal and gastric cancer samples and also between normal gastric epithelial and gastric cancer cell lines. Functionally, overexpression of miR-490-3p could reduce the growth of gastric cancer cells but not normal stomach epithelial cells. Furthermore, this inhibitory action was through the induction of autophagy. Collectively, our findings reveal the tumor suppressive function of miR-490-3p in gastric cancer for the first time and establish an important link between miR-490-3p and autophagy in gastric cancer development.

Title(C14): Dihydrotanshinone induced apoptosis in colon cancer cells by p53-independent but ROS dependent pathway

L. Wang, J.H.K. Yeung, W.Y.W. Lee, X.L. Zhou, T. Hu, C.H. Cho

Program: PhD; Supervisor: CHO Chi Hin

Dihydrotanshinone (DHTS) is one of the major tanshinones. It has been reported to have potent anti-cancer activity. However, the anti-cancer action and its underlying mechanisms on colon cancer are still undefined. In this study, the cytotoxity of DHTS was measured in colon normal and cancer cells by MTT assay. Apoptotic activity of DHTS was evaluated by FCM. Results showed that DHTS produced a selective cytotoxity against colon cancer cells through induction of apoptosis. The IC50 values for cell viability were similar between HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells (0.98±0.11μM vs 1.39±0.32μM, respectively). Furthermore the concentrations to induce apoptosis by DHTS were also similar in these two cell lines (18.57%±0.16% vs 20.52%±1.3%). All these findings suggested that the cytotoxity through induction of apoptosis by DHTS was p53 independent in colon cancer cells. In a separate experiment, pretreatment with N-Acety-L-Cysteine (NAC) or catalase-PEG, both known to be free radical scavengers, decreased apoptosis induced by DHTS. DHTS produced a dose-dependent effect on PARP cleavage by Western blot, which was also inhibited by pretreatment with NAC. We also found that DHTS produced a time- and dose-dependent increase in intracellular Ca^{2+} and ROS generation by FCM and laser confocal microscopy assays. Pretreatment with BAPTA-AM (a Ca^{2+} chelator) or catalase-PEG (a hydrogen peroxide inhibitor) prevented the increase of both intracellular Ca^{2+} and ROS generation. In conclusion, apoptosis induced by DHTS is p53 independent but ROS dependent. Increase of intracellular Ca^{2+} also plays an important role in the induction of apoptosis by DHTS in colon cancer cells.

Title(C15): Proteomic research on Hpn-induced HCC apoptosis

W.M. Wang, J.F. Zhang, H. Wang and H.F. Kung

Program: PhD; Supervisor: KUNG Hsiang Fu

Helicobacter pylori is a Gram-negative bacterium that colonizes the stomach and causes gastritis and peptic ulcerations. Hpn is a histidine-rich protein abundant (28 residues of total 60 amino acids) in this bacterium and forms oligomers in physiologically relevant conditions. Biophysical characterization shows that Hpn can bind five Ni^{2+} ions per monomer at pH 7.4, suggesting a role of Hpn in Ni^{2+} storage and homeostasis in *H. pylori*. Recent study reveals that Hpn can inhibit the proliferation of gastric epithelial AGS cells through cell cycle arrest in the G2/M phase, which may be closely related to the disruption of mitochondrial bioenergetics.

In our study, Hpn can suppress HCC cell growth. Hoechst-PI staining and flow cytometry analysis indicated that Hpn induced apoptosis in HCC cells. A two-dimensional (2D) gel electrophoresis and mass spectrometry-based comparative proteomics were performed to find the molecular mechanism of Hpn-induced apoptosis in HCC cells. Fifteen differentially expressed proteins were identified. Quantitative RT-PCR and western blot were applied to confirm the 2D results. The most interesting candidate protein will be further investigated by gain- and loss-of-function studies.

Title(C16): Regulatory role of an orphan nuclear receptor LRH-1 in castration resistant prostate cancer

L.J. Xiao, F.L. Chan

Program: PhD; Supervisor: CHAN Leung Franky

It is generally believed that the relapse of castration-resistant prostate cancer (CRPC) that develop after androgen deprivation therapy(ADT) of primary prostate cancer is mostly mediated by reactivation of the androgen receptor (AR) signaling. Several hypotheses underlie the occurrence of CRPC, such as the selection of androgen-independent clonal cell populations, aberrant splicing caused ligand-independent AR activation, AR hypersensitivity due to AR overexpression, ligand promiscuity by AR mutations and intratumoral conversion of adrenal androgens to high affinity AR ligand DHT. On the other hand, the *de novo* steroid synthesis from cholesterol in CRPC has also been proposed. In our preliminary study, we found that a nuclear receptor Live Receptor Homologue-1(LRH-1) may contribute to the *de novo* steroid synthesis through regulation expression of several critical enzymes in steroidogenesis such as CYP17A1, HSD3B1, HSD3B2 and StAR *etc*. In androgen-dependent prostate cancer cell line LNCaP, LNCaP-LRH-1 clones were more resistant to anti-androgen bicalutamide at different concentrations and also to steroids-depeleted medium. In SCID mice, tumor formed from LNCaP -LRH-1 cells can keep growing after castration, more importantly, the intratumoral androgen(Testosterone and DHT) concentrations were significantly higher in tumor tissues formed from LNCaP -LRH-1 cells than that from LNCaP-vector cells. Treatment LNCaP-LRH-1 cells with CYP17A1 inhibitor abiraterone can restore the sensitivity of LNCaP -LRH-1 cells to the anti-androgen bicalutamide and the steroids-depeleted medium. However, as to the androgen-independent prostate cancer cell line DU145, although the CYP17A1 transcripts were also increased in DU145-LRH-1 clones, overexpression LRH-1 did not significantly affect its growth features likely due to its androgen-independent property. Together, our preliminary results showed that LRH-1 may act as an important regulator in *de novo* steroid synthesis from cholesterol in prostate cancer cells and hence may play a role in advanced prostate cancer.

Title(C17): Metabolic conversion of pyrrolizidine alkaloid N-oxides to pyrrolizidine alkaloids in both liver and intestine

M.B. Yang, G. Lin

Program: PhD; Supervisor: LIN Ge

Pyrrolizidine alkaloids (PAs) and PA *N*-oxides are a group of toxins that widely exist in more than 6000 plants. Although content of PA *N*-oxides in plants are often equal or even higher than that of PAs, their intoxications are largely unknown. In our previous study, clinical cases of HSOS (hepatic sinusoidal obstruction syndrome) are found in several patients who consumed PA *N*-oxide-containing herbs, and both PA *N*-oxides and the corresponding PAs were further confirmed to exhibit similar hepatotoxicity in mice. It is well-known that PAs exert hepatotoxicity via cytochrome P450-mediated metabolic activation. Therefore, we propose that PA *N*-oxides may be metabolically converted to the corresponding PAs followed by the same metabolic activation, leading to hepatotoxicity. The current study aims to unveil the metabolic conversion of PA *N*-oxides to PAs in both liver and intestine.

Sub-cellular fraction including S9, microsome and cytosol prepared from rat liver and intestine were used for *in vitro* metabolism of a representative hepatotoxic PA *N*-oxide, riddelliine *N*-oxide (200 μM). The results demonstrated that PA *N*-oxide reduction to the PA occurred in both liver and intestine. The highest conversion rate was found in hepatic microsome (22.4±1.4 μM PA/1 hour), while hepatic cytosolic fraction contributed to a less extent (7.6±0.1 μM PA/1 hour). Reductions in both organs were all NADPH-dependent. Moreover, pyrrole-protein adducts, which directly relate to hepatotoxicity, were also determined in hepatic incubations, indicating that the reduction of PA *N*-oxide to PA might induce toxicity.

This study revealed, for the first time, that reduction of PA *N*-oxide occurred in both liver and intestine. PA *N*-oxide intoxication is probably due to such biotransformation followed by the same metabolic activation of PA in the body.

Title(C18): Inhibitory effects of lactoferrin derivatives on Candida albicans which causes infections in diabetics

C.M. Yin, J.H. Wong, T.B. Ng, J. Xia and M.M. Hui

Program: MPhil; Supervisor: NG Tzi Bun

Diabetes mellitus, specifically type 2 diabetic mellitus (T2D), is a major public health issue. The diabetic population is expected to increase from 171 million in 2000 to 366 million by 2030. Candida is frequently encountered in urinalysis studies in DM patients. Patients with T2D are at increased risk for *Candida* infection. Lactoferricin (14 amino acids) and lactoferampin (17 amino acids) were effective in prophylaxis against infections with *Candida albicans*. Another growing problem of fungal resistance to traditional drugs necessitates a search for new antimicrobial agents. The unique mechanism of action and safety profile of antifungal peptides such as lactoferricin and lactoferrampin make them appealing candidates for simultaneous or sequential use in different cases of infections.

In this project, two derivatives of lactoferrin, lactoferricin and lactoferrampin, were chemically synthesized by Fmoc solid-phase peptide synthesis. The MIC of lactoferricin and lactoferrampin against *C. albicans* (SC5134) were 0.3 mg/ml and 0.5 mg/ml, respectively. The peptides (at final concentration of 0.1 mg/ml for lactoferricin and 0.3 mg/ml for lactoferrampin) mediated membrane permeabilization as witnessed by the enhancement in fluorescence due to uptake of the dye SYTOX green. Reactive oxygen species was detectable in *C. albicans* cells after treatment with the derivatives but not in the untreated cells. Currently available evidence indicates that these derivates of lactoferrin are well tolerated in pre-clinical tests and thus they are candidates for further explorations in treating DM infections. Coating of dental implants or catheters with these peptides might prevent infections in DM patients with oral and renal symptoms.

Title(C19): Cathelicidin is a host defense peptide in controlling Helicobacter pylori survival and infection

L. Zhang, J. Yu, W.K.K. Wu, C.H. Cho

Program: PhD; Supervisor: CHO Chi Hin

Cathelicidin, a host defense antibacterial peptide in humans can eradicate different kinds of microbial infection. However, its role in *Helicobacter pylori* (*H. pylori*) infection and inflammation remains unexplored. This study sought to elucidate the actions of cathelicidin in protection against *H. pylori* infection and its associated gastritis *in vivo*. mCRAMP (a mouse cathelicidin) wild-type ($Cnlp^{+/+}$) and knockout ($Cnlp^{-/-}$) mice were given orally once every two days with mCRAMP-encoded *L. lactis* for two weeks before *H. pylori* challenge. This treatment continued for a further two months after *H. pylori* infection. Animals were then sacrificed and stomachs were excised for assessment of *H. pylori* infection and inflammatory responses in stomachs. To examine the direct antibacterial action of cathelicidin against *H. pylori*, bacteria survival was determined after exposure to a range of concentrations of the peptide prepared *in vitro*. Results showed that oral administration of *L. lactis* could survive and harbor in the gastric mucosa. In the absence of mCRAMP, $Cnlp^{-/-}$ mice exhibited stronger *H. pylori* colonization together with higher inflammation score with marked tumor necrosis factor (TNF-α), interleukin-6 (IL-6) and interleukin-β (IL-1β) expressions in the gastric mucosa when compared to the wild type mice. Furthermore in $Cnlp^{+/+}$ mice, *H. pylori* infection stimulated gastric epithelium- and neutrophil-derived mCRAMP production but not in $Cnlp^{-/-}$ animals. This finding could partially explain why there was less bacterial infection and inflammation in wild type mice. To further confirm this phenomenon, pre-treatment with mCRAMP-encoded *L. lactis* significantly increased mucosal mCRAMP level in both types of animals and reduced *H. pylori* infection and also pro-inflammatory cytokines mRNA expressions in the stomachs. Furthermore *in vitro* study showed that exposure to exogenous mCRAMP or mCRAMP-encoded *L. lactis* reduced *H. pylori* survival dose dependently. Collectively, these findings indicate that cathelicidin plays a significant role as a potential natural antibiotic for *H. pylori* clearance and prevention of gastritis. The food-grade probiotic encoded with cathelicidin is perhaps a promising biological preparation for *H. pylori* infection and its associated gastric disorders in humans.

Title(C20): Small molecule activators of microRNA-34a with anti-cancer activities identified through library screening

Z.G. Xiao, C.H. Li, Y.X. Zhu, H.K. Cheng, Y.C. Chen

Program: PhD; Supervisor: CHEN Yang Chao

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 we named compound 1 and compound 3 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. They also induced HCC cell cycle arrest and inhibited angiogenesis, Furthermore, compound 1 and compound 3 dramatically inhibited tumor growth in xenografted HCC mouse model. Meanwhile, these two compounds can activate the expression of mir-34a in HCC cells which contain wild type or mutated p53 (HepG2, Huh7, Bel7404, PLC) but not in p53 deleted cell (Hep3B), indicated that this two compounds acts as mir-34a activators in a p53 (WT or mutant) regulated pathway.

Neuro-degeneration, -development and Repair

Title of Poster Presentation	Name	Abstract No.
Characterization of miRNA-210 in regulating 3T3-L1 adipogensesis	W.C. Liang	N1
The glucagon-like peptide1 receptor agonist, exendin-4, induces emesis and increases blood pressure in the ferret	Z.B. Lu	N2
The expression of glutamine synthetase in isolated dorsal root ganglion cells in response to glutamate	K.H.Tse	N3
Donepezil shifted dominant frequency to bradygastric range and caused fluctuation in tachygastric range in ICR mice of different age groups	H.C. Wang	N4
Anthraquinones from Rheum Palmatum Inhibit Acetylcholinesterase Activity in Vitro	Y. Wang	N5
Glycine Receptor Mediated Inhibitory Currents in Rat Globus Pallidus	L.H. Xu	N6
Increased susceptibility of α-synuclein over-expressed Drosophila to iron exposure in the pathogenesis of Parkinsonism	Z.J. Zhu	N7

Title(N1): Characterization of miRNA-210 in regulating 3T3-L1 adipogensesis

W.C. Liang, Y. Wang, M.M.Y. Waye

Program: PhD; Supervisor: WAYE Miu Yee Mary

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.

Title(N2): The glucagon-like peptide1 receptor agonist, exendin-4, induces emesis and increases blood pressure in the ferret

Z.B. Lu, G. Lin, D.T.W. Yew, J.A. Rudd

Program: PhD; Supervisor: RUDD John Anthony

Glucagon-like peptide-1 receptor agonists are being developed for the treatment of type 2 diabetes and obesity, but their use is sometimes associated with nausea and emesis. In the present studies, we investigate the potential emetic mechanism of the GLP-1 receptor agonist, exendin-4.

A guide cannula was implanted into the 3rd ventricle of the ferret's brain under general anesthesia. Seven days later, two biopotential wires of a telemetric transmitter were implanted in the antrum and its pressure catheter was implanted in the abdominal aorta under general anesthesia. After a further 7 days of recovery, telemetric baseline recordings were made for 12 h before administration of exendin-4 (10 nmol, i.c.v.) or saline (15 μl, i.c.v.). Behavior and telemetric recordings were then continued for 24 h

Exendin-4 at 10 nmol, i.c.v., induced 70.4 ±40.4 retches and 12.2 ±7.7 vomits, following a median latency of 36.6 min; 5 out of 8 ferrets responded ($p < 0.05$). Exendin-4 did not affect gastric myoelectric activity (GMA), but it inhibited food intake. In the saline group, the GMA dominant frequency decreased at t=5, 6, 8 h, with an increase in bradygastria and decrease in normalgastria ($p < 0.05$). Exendin-4 elevated blood pressure significantly (systolic, diastolic, pulse, and mean blood pressure) for up to 24 hours ($p<0.05$). Exendin-4 also increased heart rate and decreased heart rate variability for up to 4 hours after injection ($p < 0.05$). Exendin-4 had no significant effect on the body temperature ($p > 0.05$).

This study showed that centrally administered exendin-4 has several adverse effects in the ferret, which may underlie the importance of GLP-1 receptors in emesis control and in the regulation of cardiovascular function.

Title(N3): The expression of glutamine synthetase in isolated dorsal root ganglion cells in response to glutamate

K.H. Tse, K.B.S. Chow, and H. Wise

Program: PhD; Supervisor: WISE Helen

In the dorsal root ganglion (DRG), sensory neurons are enveloped by glutamate synthetase (GS)-expressing satellite glial cells (SGCs). SGCs operate a glutamate-glutamine shuttle with sensory neurons by active uptake of neuronally released glutamate and its conversion to glutamine by GS. The present study aims to investigate the expression of GS in isolated DRG cells and examine its regulation by exogenous glutamate

DRG cells cultures (mixed cells) were prepared from adult SD rats, along with neuron-enriched (N-cells) and glial cell (G-cell) cultures. The 3 cell groups were cultured for periods up to 5DIV, then GS mRNA was quantified by real time PCR using TaqMan® probes. GS mRNA expression was determined relative to β-actin mRNA expression. After 1 DIV, glutamate (0.1-10 mM) was added to mixed cells for 24 h, then mRNA was extracted, the viability of the cells was determined by MTT assay, and GS was visualized using confocal microscopy. To demonstrate if the changes in GS mRNA expression and the cell viability were induced through glutamate receptors, antagonists against NMDAR (MK801), Kainate/AMPAR(CNQX), mGluRI, II and III (CPPG and E4CPG) were employed.

A progressive loss of GS mRNA was observed in all 3 cell preparations during a 5 day period ($p < 0.05$ at 5DIV versus fresh cells), with greater loss of GS mRNA in the G-cell fraction compared to neuron-containing fractions. The addition of high glutamate (10 mM) significantly increased GS mRNA ($p < 0.05$), but also reduced cell viabilities to 50%. However, none of the glutamate receptor antagonist inhibitors prevented the reduction in cell viability or GS mRNA induction.

Our results suggest the presence of a glutamate-glutamine shuttle between sensory neurons and associated glial cells in mixed DRG cell cultures. High level of exogenous glutamate induced GS expression in glia which could be a neuroprotective response.

Title(N4): Donepezil shifted dominant frequency to bradygastric range and caused fluctuation in tachygastric range in ICR mice of different age groups

H.C. Wang, A. Lu, D. Fang, and J.A. Rudd

Program: PhD; Supervisor: RUDD John Anthony

Donepezil, an acetylcholinesterase inhibitor, is used to treat Alzheimer's disease (AD). In the present study, we investigate the *in vivo* effects of donepezil on gastric myoelectrical activity (GMA) in 3 month old (mo) and 12 mo ICR mice.

Male ICR mice (3 and 12 mo, 30-40 g) were anaesthetised and surgically implanted with telemetry devices (PhysioTel® ETA-F20, DSI, U.S.A.) with recording wires sutured into the serosal side of the stomach. After 7 days recovery, animals were randomised to receive vehicle (saline 2 ml/kg, i.p.) and donepezil (3 mg/kg; 2ml/kg, i.p.). Two hours of baseline GMA was recorded prior to vehicle/drug administration; recordings then continued for a further 6 h. Raw data were processed using Spike2 (Cambridge Electronic Design, U.K.) and analysed using a repeated measures 2-way ANOVA followed by Bonferroni t-tests. R program was used to analyse the levels of fluctuations and variability.

There were no age-related differences between the 2 h baseline data of 3- and 12-mo animals ($P > 0.05$). Saline had no effect on any of the parameters of the slow waves during the experiment ($P > 0.05$). However, donepezil reduced the DF gradually in both age groups, producing significant increases in the % power of the bradygastric range (0 to DF-1.5 cpm). Such effect seems to be more apparent in the 3mo old than 12 mo mice. The DF then shifted back to pre-donepezil levels with an increased power; dysrhythmia (fluctuation) was also observed in the tachygastric range in both age groups. The % power of normogastric (DF±2 cpm) and tachygastric range (DF+2 to 15cpm) was not affected by donepezil in any age group ($P>0.05$).

Acetylcholine levels to stimulate both nicotinic and muscarinic receptors, causing inhibitory effect followed by dysrhythmia.

Title(N5): Anthraquinoes from Rheum Palmatum inhibit Acetylcholinesterase Activity in vitro

Y. Wang, H.Q. Lin, L.S. Li and D.C.C. Wan

Program: PhD; Supervisor: WAN Chi Cheong David

Rhubarb palmatum L., locally known as Da-Huang in China, has been widely used as one traditional Chinese herbal medicine for thousands years . It is still frequently prescribed by TCM practitioners in China. Pharmacological studies have demonstrated that anthraquinones is one of the primary active ingredients of *Rhubarb palmatum* L. which exhibit various bioactivity properties. Here, we report the identification of anthraqinones as potent acetylcholinesterase (AChE) inhibitors from *Rhubarb palmatum* L. . We found that Emodin and aloe-emodin had a strong AChE inhibitory effect with IC50 21.8μM and 26.8μM, respectively. However chrysophanol and rhein showed relative weak anti-AChE activity with IC50 75.8μM and 263.6μM, respectively. We then performed ligand-receptor docking study and tried to describe the binding simulation between anthraquinones and human acetylcholinesterase. In the catalytic gorge, four favorable compounds with the best binding modes formed a π-π interaction with the indole ring of TRP86. Notably, emodin and aloe-emodin formed hydrogen bonds with the active site containing amino acids close to the TRP86; while the residues with which chrysophanol and rhein made hydrogen bond are far away from the indole ring of TRP86. TRP86 is a well-known and significant residue in human acetylcholinesterase active pocket and the indole ring of this residue always forms a π-π interaction with AChE inhibitors. According to *in vitro* results and docking simulations, it seems that hydrogen bonds adjacent to the indole ring of TRP86 might strengthen the π-π interaction between residue and inhibitors. In conclusion, we identified four anthraquinones from *Rhubarb palmatum* L. as potential AChE inhibitors and emodin and aloe-emodin exhibited strong inhibitory effects. Furthermore, docking simulation showed that anthraquinones contacted with TRP86 through a π-π interaction and the hydrogen bonds adjacent to this π-π interaction are also key factors of binding affinity.

Title(N6): Glycine Receptor Mediated Inhibitory Currents in Rat Globus Pallidus

L.H. Xu, B. Li, W.H. Yung

Program: PhD; Supervisor: YUNG Wing Ho

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

Two types of GP neurons were distinguished mainly based on the presence (type A) or absence (type B) of hyperpolarization-activated inward current. Both types of neurons were immunopositive for GlyRs, and responded to the application of both glycine and GABA by generating an outward current that inhibited the firing of these neurons. The specificity of glycine on glycine receptor was confirmed by the antagonistic action of strychnine but not picrotoxin. Interestingly, the current evoked by glycine and GABA in type A neuron was significantly higher than that evoked in type B neuron. We also find the mRNA of glycine receptor α1, α2 and GABA α1 expression in type A neuron were much higher than type B. Finally, while most of the spontaneous inhibitory postsynaptic currents (sIPSCs) were mediated by GABA-A receptors, a small portion of the sIPSCs were mediated by GlyRs. In conclusion, despite that GABA-A receptor type is the major receptor mediating the inhibitory action originating from the striatum or from local neurons, glycine and glycine receptor may play a specific role in inhibiting the neuronal activities in different subtypes of GP neurons. These findings may have implications in understanding the etiology of GP-related motor disorders.

Title(N7): Increased susceptibility of α-synuclein over-expressed Drosophila to iron exposure in the pathogenesis of Parkinsonism

Z.J. Zhu, T.Y. Tsim, Y. Ke

Program: PhD; Supervisor: KE Ya

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by selective loss of dopaminergic neurons and the presence of Lewy body inclusions in the substantia nigra of the midbrain. Although the etiology of PD remains incompletely understood, emerging evidence suggests that dysregulated iron homeostasis may be involved. α-synuclein is the major component of Lewy bodies, and point mutations of this gene are associated with familial PD. However, how dysregulation of α-synuclein leads to neurodegeneration is not entirely clear. Here, based on the Drosophila model, we tested the hypothesis that interaction between iron and α-synuclein accelerates pathogenesis of Parkinsonism. Three groups of Drosophila, including normal, wild-type α-synuclein (WT) and mutated (A53T and A30P) α-synuclein over-expressing Drosophila were fed with normal Formula 4-24 instant medium or medium added with different concentrations ferric ammonium citrate (FAC) for up to 30 days. After the treatment, they were subjected to whole-mount iron staining for brain iron content, negative geotaxis assay for locomotor function and whole-mount immunostaining for dopaminergic neurons. FAC treatment increased the brain iron contents leading to increased death rate and motor dysfunctions. These effects of iron were aggravated by overexpression of α-synuclein, and to a larger extent mutated α-synuclein. The dopaminergic neurons in clusters PPM1/2 and PPM3 in the normal, WT and mutated α-synuclein groups decreased with age in both control and FAC medium treatment groups. However, compared with the control medium group, treatment with FAC medium led to specific dopaminergic neuronal loss in cluster PPM3 after 30 days. Taken together, these data reveal that α-synuclein expressed Drosophila exhibit greater susceptibility to iron toxicity, which is exacerbated by A53T and A30P mutations.

Reproduction, Development and Endocrinology

Title of Poster Presentation	Name	Abstract No.
Functional study on the downregulated miR-199a-3p in testicular tumor	B.F. Chen	R1
Dedifferentiation-Reprogrammed Mesenchymal Stem Cells with Enhanced Tumor Tropism	R. Chen	R2
Timing and Routes of Entry of Microglial Progenitors to the Embryonic Central Nervous System	G.W.H. Chow	R3
Molecular mechanism of regulation and action of microRNA 199a in development and differentiation	S. Gu	R4
CFTR-mediated Cl- efflux contributes to membrane depolarization and bursting action potentials required for glucose-stimulated insulin secretion in pancreatic islet β-cells	J.H. Guo	R5
Activation of the novel G protein-coupled receptor 30 (GPR30) inhibited P2Y receptor-mediated Ca2+ signaling in human bronchial epithelia	M.Y. Hao	R6
Efficient targeted gene disruption in Xenopus embryos using engineered transcription activator-like effector nucleases (TALENs)	Y. Lei	R7
NDAPH oxidase is critical in regulating pancreas development and endocrine cell differentiation	J. Liang	R8
The role of Dragon in renal tubular injury	W.J. Liu	R9
Discovering novel epigenetic regulation in spermatogonial stem cell development	A.C. LUK	R10
High Glucose Represses -klotho Expression and Impairs FGF21 Action in Pancreatic Islets	W. Y. So	R11
The role of CFTR in the development of benign prostatic hyperplasia (BPH): negative regulation on COX-2/PGE2	X. Sun	R12
Ets-1 regulates neural crest formation through mediation BMP signaling pathway	C.D. Wang	R13

HFE promotes hepcidin expression by reducing the ubiquitination proteasomal degradation of the BMP type I receptor ALK3 in hepatocytes	X.G. Wu	R14
IL-6, TNF-α and IL-1β play distinct roles in inhibiting growth hormone signaling in the liver	Y.S. Zhao	R15

Title(R1): Functional study on the downregulated miR-199a-3p in testicular tumor

B.F. Chen; W.Y. Chan

Program: PhD; Supervisor: CHAN Wai Yee

It was previously demonstrated that miR-199a was downregulated in testicular germ cell tumor (TGCT) probably caused by hypermethylation of its promoter. Further study found that re-expression of miR-199a in testicular cancer cells (NT2) led to suppression of cell growth, cancer migration, invasion and metastasis. More detailed analyses showed that these properties of miR-199a could be assigned to miR-199a-5p, one of its two derivatives. The biological role of the other derivatives, miR-199a-3p in TGCT, remains largely uncharacterized. In this report we identified DNA (cytosine-5)-methyltransferase 3A (DNMT3A), the *de novo* methyltransferase, as a target of miR-199a-3p. We demonstrated that after transient transfection of miR-199a-3p into NT2 cells, DNMT3A expression (especially DNMT3A2, the DNMT3A gene isoform 2) at mRNA and protein level was significantly decreased. The dual luciferase reporter assay results showed that miR-199a-3p reduced the luciferase activity in NT2 and HEK 293T cells significantly when co-transfected with miR-199a-3p and Luc-DNMT3A construct harboring the potential binding site for miR-199a-3p. In clinical samples, DNMT3A2 was overexpressed in malignant testicular tumor, and the expression of DNMT3A2 was inversely correlated with the expression of miR-199a-3p. To further verify a potent role for the miR-199a-3p/DNMT3A pathway in mediating TGCT tumor cell survival and in regulating TGCT tumor growth, we will use miR-199-3p overexpressing stable cells and the stably transfected shDNMT3A-NT2 cells to test whether they have similar biological properties (cell proliferation, apoptosis, migration and invasion).

Title(R2): Dedifferentiation-Reprogrammed Mesenchymal Stem Cells with Enhanced Tumor Tropism

R. Chen, X.H. Zhang, J.T. Zhang, L.L. Tsang, H.C. Chan, X.H. Jiang

Program: PhD; Supervisor: CHAN Hsiao Chang and JIANG Xiao Hua

Mesenchymal stem cells (MSCs) are inherently attracted toward cancer, so they have been used as vehicles to deliver therapeutic genes for cancer treatment, including glioblastoma. However, low levels of MSC recruitment in vivo largely limit their overall effectiveness and clinical use. Therefore, development of methods for improving the homing of MSCs into tumor cells to increasing gene therapeutic effects is in highly required.

Previous study from our group demonstrated that after in vitro induction of neuronal differentiation and dedifferentiation, MSCs, which had already committed to neuronal lineage, reverted to a primitive cell population (De-neuMSCs) exhibiting a reprogrammed phenotype distinct from naïve MSCs. De-neuMSCs acquired enhanced neuron differentiation efficacy and anti-apoptosis ability. Interestingly, in this study also found De-neuMSCs showed enhanced migration ability toward various kinds of cancer cells including a glioma cell line, U87. Of note, a large number of chemokines and inflammation factors were highly expressed in De-neuMSCs which might contribute to the enhanced migration ability observed in De-neuMSC.

Our study could provide a new way to improve gene therapy effect in cancer treatment.

Title(R3): Timing and Routes of Entry of Microglial Progenitors to the Embryonic Central Nervous System

G.W.H. Chow, T.C. Ng, P. Rezaie, W.Y. Chan

Program: MPhil; Supervisor: CHAN Wood Yee

Microglia are resident macrophages within the central nervous system (CNS). Their embryonic origins and routes of entry to the CNS are still not clear. In this study, we used Iba1 (ionized calcium binding adaptor molecule 1) as a specific marker to label microglial progenitors in mouse embryos, neural tube culture and multiple immunohistochemical localization to establish their routes of entry to the CNS. Iba1+ microglial progenitors were found in the mesenchyme near the neural epithelium at E9.5, and appeared within the neural tube and the liver at E10.5. The number of Iba1+ cells in the embryo increased dramatically from E9.5 to E11.5. By E13.5, significant numbers of Iba1+ cells were observed in the mantle layer. Examination of sectioned tissues suggested that Iba1+ cells entered the neural tube through its basal surface, apical surface or blood vessels. When the neural tube at E9.5 was isolated to cultured for 1 day, no Iba1+ cells were observed following immunohistochemical localiztion. These results may implicate that microglial progenitors do not seed the neural tube before E9.5, and also suggest that cells derived from the neural epithelium do not contribute to the microglial progenitor pool. In conclusion, microglial progenitors expressing Iba1 enter the neural tube through multiple sites, and microglial progenitors seed the CNS at specific time frame while the neural epithelium is not a source for microglial progenitors.

Title(R4): Molecular mechanism of regulation and action of microRNA 199a in development and differentiation

S. Gu, W.Y. Chan

Program: PhD; Supervisor: CHAN Wai Yee

MicroRNA-199a (miRNA-199a) has been shown to behave quite differently in different systems and diseases. It is encoded by two loci in the human genome, miR-199a-1 on chromosome 19 and miR-199a-2 on chromosome 1. Both loci give rise to the same miRNA. The cause of the diverse action of the miRNA is not clear. However, it is likely caused by different regulations of the two genomic loci and variable targets of the miRNA in different cells and tissues. Promoter methylation has been shown to be responsible for suppressing the expression of miR-199a in testicular germ cell tumors (TGCTs) for both miR-199a-1 and -2. On the other hand, in glioma a change in promoter methylation occurred only in miR-199a-2, while that of miR-199a-1 did not change indicating that alteration in promoter methylation might be disease- or tissue-specific. Besides promoter methylation, transcription factor(s) also played important roles in controlling the expression of miR-199a. REST had been reported to bind to the promoter of both miR-199a genes, raising the possibility that it might be involved in controlling the expression of the miRNA. The action of miR-199a was also affected by its downstream targets expressed in the cells or tissues. We had applied both functional genomic and proteomic approaches to study the targets of miR-199a in different cell models. By studying the mechanisms that control the expressions of miR-199a and its various downstream targets in different systems, we hope to use miR-199a as a model to illustrate the complexity of miRNA biology.

Title(R5):CFTR-mediated Cl- efflux contributes to membrane depolarization and bursting action potentials required for glucose-stimulated insulin secretion in pancreatic islet β-cells

J.H. Guo, H. Chen, X.L. Zhang, H.C. Chan

Program: PhD; Supervisor: CHAN Hsiao Chang

Glucose-stimulated insulin secretion is associated with a complex electrical activity in the pancreatic islet β-cell, which is characterized by a slow membrane depolarization superimposed with bursts of action potentials. Closing ATP-sensitive K^+ channels (KATP) in response to glucose increase is generally considered the initial event that depolarizes the β-cell membrane and activates the voltage-dependent Ca^{2+} channels, which constitutes the major depolarizing component of the bursting action potentials giving rise to the cytosolic calcium oscillations that trigger insulin release. While Cl^- has been implicated in an unknown depolarization current of the β-cell, the responsible Cl^- channel remains unidentified. Here we show functional expression of the cystic fibrosis transmembrane conductance regulator (CFTR) and its activation by glucose in the β-cell. The glucose-elicited whole-cell currents, membrane depolarization, electrical bursts (both magnitude and frequency), Ca^{2+}oscillations and insulin secretion could be abolished or reduced by inhibitors/knockdown of CFTR in primary mouse β-cells or RIN-5F β-cell line, or significantly attenuated in isolated mouse islet β-cells from CFTR mutant mice compared to that of wildtype. Significantly increased blood glucose level accompanied with reduced level of insulin is found in CFTR mutant mice compared to the wildtype. The present results reveal a previously unrecognized important role of CFTR in glucose-stimulated insulin secretion.

Title(R6): Activation of the novel G protein-coupled receptor 30 (GPR30) inhibited P2Y receptor-mediated Ca2+ signaling in human bronchial epithelia

M.Y. Hao, A.W.M. Chow, W.C.Y. Yip, J.M.L. Yuen, L.X. Sun, C.H.K. Cheng and W.H. Ko

Program: PhD; Supervisor: KO Wing Hung

The airway epithelium plays a central role in respiratory physiology through its transporting and immunological functions. Our previous study suggests that P2Y receptors are expressed in airway epithelia and play significant role in regulating transepithelial ion transport. P2Y receptors belong to the family of purinergic receptors, which can be stimulated by nucleotides such as UTP and UDP. P2Y receptors are G protein-coupled receptors and classically signal through Gq, resulting in an increase of intracellular Ca2+concentration and thereby activation of Ca2+-dependent ion channels and downstream signaling pathway(s).In addition, P2Y receptors may be involved in asthmatic inflammation.

Estrogen (or E2) is an important hormone in human physiology that regulates multiple aspects of biological processes. In addition to the classical nuclear hormone receptors ERα and ERβ, a novel estrogen receptor, G-protein coupled receptor 30 (GPR30), was recently identified and found to be involving in both rapid signaling and transcriptional regulations. The GPR30 action is unclear but it has been implicated in mediating anti-inflammatory response. This study aims to investigate the pro-inflammatory action of P2Y receptors and the interaction between GPR30 and P2Y receptor-mediated signaling pathways.

In this study, a human bronchial epithelial cell line, 16HBE14o-, derived from human bronchial surface epithelial cells was used. In some experiments, primary normal human bronchial epithelial cells were also used (ScienCell Research Laboratories, San Diego, CA, USA). Data in our study suggest that GPR30 is expressed on human airway epithelia and activation of GPR30 by E2 or its specific agonist, G1, inhibited the P2Y receptor-mediated Ca2+ signaling pathway. Therefore, the anti-inflammatory role of GPR30 may be due to its opposing effect on the pro-inflammatory pathway activated by the P2Y receptors in inflamed airway epithelia.

Title(R7): Efficient targeted gene disruption in Xenopus embryos using engineered transcription activator-like effector nucleases (TALENs)

Y. Lei, X.G. Guo, Y. Liu, Y. Cao, Y. Deng, X.F. Chen, C.H.K. Cheng, I.B. Dawid, Y.L. Chen and H. Zhao

Program: PhD; Supervisor: ZHAO Hui

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

Title(R8): NDAPH oxidase is critical in regulating pancreas development and endocrine cell differentiation

J. Liang, Q. Cheng, K.K. Leung and P.S. Leung

Program: PhD; Supervisor: LEUNG Po Sing

NADPH oxidase is a pivotal reactive oxygen species-producing enzyme, which is involved in the cell development, regeneration and differentiation. Nonetheless, the potential role of NADPH oxidase in regulating pancreas development and endocrine cell lineage remains unclear. This study is aimed to investigate the expression and function of NADPH oxidase during pancreas development and in endocrine cell differentiation using mouse embryonic pancreas and human pancreatic progenitor cell (PPC) models, respectively. Our PCR results showed that NOX4 and P22, the subunits of NADPH oxidase, were expressed during embryonic pancreas development but their expression disappeared in neonatal pancreas; the expression levels of NOX4 and P22 reached a peak at E15.5 and declined until neonate pancreas. NOX4 expression was localized to beta-cells, acinar-cells and ductal-cells at E15.5, while its expression was decreased in acinar-cells and ductal-cells at E17.5, concomitant with an increase in $Insulin^{+}NOX4^{+}$ cells, as evidenced by immunocytochemistry; NOX4 also had a high expression at E15.5 with NGN3 positive cells, a marker of pancreatic progenitor cells. On the other hand, *ex vivo* treatment of DPI, a NADPH oxidase inhibitor, reduced the expression levels of endocrine cell differentiation markers (NGN3, PAX4 and NKX6.1) and mature islet cell markers (insulin and glucagon) in embryonic pancreas. Interestingly, immunocytochemical analysis showed a low proliferative capacity in embryonic pancreatic cells treated with DPI; DPI also decreased the expression of SOX9, a transcription factor of NGN3, at both mRNA and protein levels. In corroboration with the mouse embryonic pancreas, our *in vitro* human PPC system revealed that expression of NOX4 and P22 levels were enhanced during the differentiation of human PPCs into islet-like cell clusters (ICCs). Our data indicate that NADPH oxidase has a critical role in regulating pancreas development and endocrine cell differentiation, which is probably via mediation of SOX9-NGN3 expression.

Title(R9): The role of Dragon in renal tubular injury

W.J. Liu, Y. Xia

Program: PhD; Supervisor: XIA Yin

Repulsive guidance molecule b (RGMb/Dragon) acts as a co-receptor for bone morphogenetic proteins (BMPs). BMP signaling plays an important role in kidney injury and repair. Our previous studies have shown that Dragon is highly expressed in renal tubular epithelial cells. However, the function of Dragon in the kidney remains unclear. We now have results showing that Dragon overexpression increased cell death in mouse inner medullary collecting duct (mIMCD3) cells cultured in hypoxia, accompanied with increased levels of cleaved PARP. Knockdown of Dragon inhibited IL-6 expression in mIMCD3 cells. Dragon expression was upregulated in the kidneys subjected to unilateral ureteral obstruction (UUO) compared with the control kidneys, in both wild-type mice and Dragon heterozygous knockout (Dragon +/-) mice. Interestingly, Dragon +/- mice showed less epithelial cell apoptosis and less tubular injury after UUO. We also found that, compared with wild type mice, Dragon +/- mice subjected to ischemia-reperfusion injury of the kidney showed decreased levels of a number of inflammatory factors including IL-6, TNF-α, IL-1β, MCP-1 and MIP-2 in the kidney. Therefore, our results suggest that Dragon promotes inflammation and induces epithelial apoptosis, thus contributing to kidney injury.

Title(R10): Discovering novel epigenetic regulation in spermatogonial stem cell development

A.C. LUK, S.H. NG, H.Y. GAO, L. LI, J.J. TU, W.Y. CHAN, T.L. LEE

Program: PhD; Supervisor: LEE Tin Lap and CHAN Wai Yee

Parental aging is a global problem that accounts for approximately 50% of infertile couples. Majority of observations on paternal aging were viewed from traditional genetic and regulation studies, but seemed incapable to generate the whole picture. Here we would like to examine the effect of a novel epigenetic regulation on paternal aging and other developmental regulators in male reproduction through studying the progenitor cell of sperm production, known as spermatogonial stem cells (SSC).

Previous epigenetic studies focused mainly on 5-methylcytosine (5mC). Recently, emerging evidence suggested that 5-hydroxymethylated cytosine (5hmC) is another functional epigenetic modification in eukaryotic genome. Its regulatory effects on differentiation of neurons and embryonic stem cells have been widely demonstrated in recent studies. However, the roles of 5hmC in the biology of SSC remain unexplored.

We hypothesize that hydroxylmethylation of genomic cytosine plays important regulatory roles in the development of SSCs. Through a novel bioinformatics pipeline, we analyzed a set of RNA-seq and 5hmC-seq data, and identified a list of genes with both 5hmC-enrichment in promoter regions and specific expression pattern over different spermatogonial differentiation stages for further *in vitro* and *in vivo* validation.

In addition, our preliminary data demonstrated a consistent decreasing trend of expression of ten-eleven translocation (Tet) protein family, which are capable to convert genomic 5mC to 5hmC, after 12 passages in stem cell-like C18-4 cells (mouse type A spermatogonia), suggesting potential correlation between aging and 5hmC epigenetics. Moreover, we showed a higher expression level of Tet in stem-cell like C18-4 cells than in somatic TM4 (mouse Sertoli) cell. Our dot-blot results also demonstrated a higher genomic 5hmC content in C18-4 than in TM4 cells, implying possible stemness-related regulation of 5hmC.

Taken together, these data revealed a potential regulation of 5hmC/Tet in SSC aging and development, which furthered our knowledge on male reproductive health and infertility control.

Title(R11): High Glucose Represses -klotho Expression and Impairs FGF21 Action in Pancreatic Islets

W. Y. So, L. H. Chen, Q. Cheng and P. S. Leung

Program: PhD; Supervisor: LEUNG Po Sing

Fibroblast growth factor (FGF) 21 is a distinctive member of the FGF family whose actions require the cofactor-klotho. FGF21 has been demonstrated to normalize glucose, lipid and energy homeostasis in several disease models including diabetes and obesity. Restricted expression of -klotho in liver, pancreas and adipose tissue provides the mechanistic basis for tissue-specific actions of FGF21 and thus implicates the important roles of -klotho in these tissues. Previous studies have shown that type 2 diabetes (T2DM) maybe a state of FGF21 resistance; meanwhile, pancreas is one of the target tissues of FGF21. Therefore, we aimed to examine the roles of -klotho and the actions of FGF21 in pancreatic islets during the pathogenesis of T2DM and the underlying mechanisms in this study.

Pancreatic islets were isolated from db/db mice and their lean littermates. mRNA expression was evaluated by real-time RT-PCR; protein expression and post-receptor signaling pathways were analyzed by Western blotting.

Our results showed that the expression of -klotho was reduced in db/db mice islets in an age-dependent manner. FGF21-induced phosphorylation of FGF receptor substrate (FRS) was impaired in islets from overt diabetic mice. In corroboration, *exvivo* studies revealed that high glucose concentrations down-regulated -klotho expression in islets and reduced FGF21-induced FRS phosphorylation. In addition, rosiglitazone, an anti-diabetic drug, reversed the down-regulation of -klotho in both db/db mice islets and islets treated with high glucose conditions.

Our data suggest that pancreatic islets of overt diabetic mice are less responsive to FGF21 which maybe due, partly, to the down-regulation of -klotho induced by hyperglycemia. Rosiglitazone, which is protective against hyperglycemia, prevents -klotho repression in islets under high glucose conditions. These findings highlight the involvement of glucose in the negative regulation of -klotho expression and FGF21 action, thus formulating a novel pathophysiological role of FGF21 in pancreatic islets in patients with T2DM.

Title(R12): The role of CFTR in the development of benign prostatic hyperplasia (BPH): negative regulation on COX-2/PGE2

X. Sun, C. Xie, J. Chen, X.H. Jiang, H.C. Chan

Program: PhD; Supervisor: CHAN Hsiao Chang

Benign prostatic hyperplasia (BPH) is an aging related disease with hyperplasia of prostatic stromal and epithelial cells, resulting in the formation of large, discrete nodules in the periurethral region of the prostate. While it is likely that multiple factors are causally related to the development of BPH, the pathogenesis of BPH remains incompletely understood. In this study, we aimed to determine the role and underlying mechanisms of CFTR in the development of BPH. We found that the expression of CFTR was decreased whereas the expression of COX2 was up-regulated as rat aging. In addition, we demonstrated that inhibition or knockdown of CFTR in CFTR expressing-PNT1A cells promoted NF-κB nuclear translocation, COX2 expression and PGE2 production. Interestingly, PGE2 did not have any effect on PNT1A cell growth, but it could promote stromal cell growth. The conditional medium of CFTR inhibition and knockdown-PNT1A cells could promote stroma cell proliferation which could be reversed by NF-κB or COX2 inhibitors. Given that PGE2 stimulates stromal cell proliferation but not epithelium cells, it is plausible that down regulation of CFTR in prostate epithelial cells induces COX-2/PGE2-mediated paracrine stimulation of stroma over growth. In consistence with *in vitro* data, we found that CFTR expression was markedly lower in human hyperplasia prostate gland as compared to the normal gland whereas the NF-κB nuclear translocation and COX2 expression was higher in BPH gland. Taken together, these results indicate a role of CFTR in regulating PGE2 release in the prostate gland, defects of which may contribute to the development of BPH.

Title(R13): Ets-1 regulates neural crest formation through mediation BMP signaling pathway

C.D. Wang and H. Zhao

Program: PhD; Supervisor: ZHAO Hui

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 immune cell development. 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 was 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. Overexpression of ets-1 up-regulated neural markers, sox2 and zic1, but down-regulated epidermis marker, epidermis keratin. We found overexpression of ets-1 attenuated BMP signaling and its target gene, Id3. Our study showed that Ets1 repressed NC formation through binding to HDAC1, and inhibition of the interaction between Ets1 and HDAC1 enabled Ets1 to induce NC. 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.

Title(R14): HFE promotes hepcidin expression by reducing the ubiquitination proteasomal degradation of the BMP type I receptor ALK3 in hepatocytes

X.G. Wu, Y. Wang, W.H. Cheng, Y. XIA

Program: PhD; Supervisor: XIA Yin

HFE mutations result in reduced expression of hepcidin, an antimicrobial peptide produced in the liver, which also negatively regulates iron in circulation by controlling iron absorption from dietary sources and iron release from macrophages. However, the mechanism by which HFE regulates hepcidin expression is unknown. Recently, we have demonstrated that the bone morphogenetic protein (BMP) pathway is central for hepcidin regulation in hepatocytes. We hypothesize that HFE regulates hepcidin expression in the liver through the BMP signaling pathway.

Immunoprecipitation experiments were performed on COS7, HepG2 and Hep3B cells to investigate the interaction of HFE and ALK3. Real-time PCR was used to examine the expression of hepcidin mRNA in the presence of HFE alone or HFE in combination with ALK3. Ubiquitination assays were performed to examine the effects of HFE on ALK3 ubiquitination.
Results: 1.HFE induced phospho-Smad1/5/8 levels and hepcidin expression in Hep3B cells. 2. HFE interacted with ALK3 but not ActRIIA. 3. HFE stabilized ALK3 in a dose dependent manner. 4. HFE inhibited the ALK3 degradation by ubiquitin-proteasome pathway. 5. HFE and ALK3 stimulated hepcidin expression synergistically.

Our results suggest that HFE promotes hepcidin expression through interacting with ALK3 to inhibit its degradation.

Title(R15): IL-6, TNF-α and IL-1β play distinct roles in inhibiting growth hormone signaling in the liver

Y.S. Zhao, Y. Xia

Program: PhD; Supervisor: XIA Yin

Protein hypercatabolism and muscle wasting characterize catabolic states induced by inflammatory diseases, such as juvenile idiopathic arthritis and Crohn's disease. Evidence suggests that during inflammation, the liver becomes resistant to growth hormone (GH) actions, leading to downregulation of an anabolic gene IGF-I and activation of catabolic process. Decades of studies demonstrated that proinflammatory cytokines IL-6, TNF-α and IL-1β are involved in the pathogenesis of hepatic GH resistance via mechanisms including the downregulation of the GH receptor (GHR), and the upregulation of suppressor of cytokine signaling-3 (SOCS3). However, the relative importance of and the differential mechanisms for these individual cytokines in regulating GH signaling are not fully understood. Using Huh-7 hepatoma cells and chronic and acute mouse models of chronic inflammation, we now show that TNF-α and IL-1β but not IL-6 inhibited hepatic GHR expression, and that IL-6 was the predominant inducer of hepatic SOCS3 expression. When GHR expression was blocked, removal of IL-6 and SOCS3 did not restore hepatic IGF-I expression in mice while removal of TNF-α and IL-1β increased GHR and IGF-I expression. Our results suggest that TNF-α/IL-1β and IL-6 inhibit GH signaling at the receptor and postreceptor levels respectively, and that IL-6 action can be overridden by TNF-α, IL-1β and perhaps some other factors.

Stem Cells and Regeneration

Title of Poster Presentation	Name	Abstract No.
Functional study of Smad7 in bone development and BM-MSCs characterization	N. Li	S1
The role of erythropoietin and erythropoietin receptor in chondrogenesis	L. Wan	S2
The Research of Stem Cell Secretion on Differentiation of MSC	K.X. Wang	S3
Function of the BRE gene in spermatogenesis	Y. Yao	S4

Title(S1): Functional study of Smad7 in bone development and BM-MSCs characterization

N. Li, G. Li

Program: PhD; Supervisor: LI Gang

TGF-β controls proliferation, differentiation, apoptosis and other functions in most cell types during embryonic and postnatal life. Smad7 has been well demonstrated to be a negative regulator of TGF-β signaling inhibits TGF-β signaling through multiple mechanisms in both the cytoplasm and nucleus. To investigate the role of the TGF-β/Smad7 signaling in the process of bone development and MSCs characterization, we performed a serious of in-vivo and in-vitro experiments using wild-type (WT) and Smad7ΔE1 mice (KO).

After the confirmation of mBM-MSCs by flow cytometry, multi-differentiation assays including osteogenesis, adipogenesis and chondrogenesis were performed to compare the characterization; specific staining as well as quantitative acetic acid extraction methods were used and the mRNA expression of relative markers were also detected by quantitative real-time RT-PCR. We also used TRAP staining and bone resorption assay to compare the osteoclastogenic potential. The parameters of long bone development at 6, 12 and 24 weeks were assessed using digital X-ray, micro-CT, compression mechanical tests and histology methods. Data analysis was performed using SPSS software and Mann-Whiney U test, $p \leq 0.05$ was regarded as statistically significant.

The adipogenic potential at day 7, 14 and 21 by Oil Red O staining (n=3) showed more and earlier lipid droplets formation, and the mRNA expression (n=6) of PPARγ and C/EBPα was significantly higher in the Smad7 KO group comparing to the control group. The osteogenic potential at day 7 and 14 using Alizarin Red S staining (n=3) and quantitation in the KO group showed less mineralized nodules, and the mRNA expression (n=6) of collagen Ⅰ and RUNX2 were also lower. The osteoclastogenic potential of the KO mBMMs was significantly elevated than that of WT mice when induced by RANKL/M-CSF and detected by TRAP staining (n=3). The mRNA expressions (n=6) of TRAP and CTR were significantly higher, and the osteoclasts were more and larger in the KO group than those in the WT group. Although no visible difference was seen between the long bones by digital X-ray among the KO and WT mice, micro-CT results showed that KO group has decreased of trabecular number (TbN), thickness (TbTh), and increased trabecular spacing (TbSp) in metaphysic region of the femurs at 6, 12 and 24 weeks (n=12) separately with statistical significance.

All the results suggest that Smad7 plays a functional role both in vitro and in vivo, and it can regulate TGF-β signaling on BM-MSCs characterization, BMMs osteoclastogenesis, and also the bone remodeling. The precise function of Smad7 on skeletal development is still unclear and needs further study, perhaps in disease models such as fracture healing and osteoporosis.

Title(S2):The role of erythropoietin and erythropoietin receptor in chondrogenesis

L. Wan, W.P. Tsang, C. Wan

Program: PhD; Supervisor: WAN Chao

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 signaling involves in the development and regeneration of several non-hematopoietic organs including heart, brain and bone, et al. It is known that EPO promotes differentiation of mesenchymal stem cells into osteoblasts, and enhances the healing process of bone fracture. However, the underlying mechanisms of EPO/EPOR signaling 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 results show that EPO and EPOR are abundantly expressed in chondrocytes of pre-hypertrophic and hypertrophic zone of the growth plate while they are weekly 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 recombinant mouse EPO indexed by BrdU incorporation assay. Alcian Blue staining for extracellular matrix proteoglycan indicates that EPO promotes the differentiation of chondrocytes. This is accompanied by upregulated chondrogenic marker genes including SOX9, SOX5, SOX6, collagen type 2, and aggrecan. SiRNA mediated knockdown of EPOR in chondrocytes has adverse effects on the differentiation of chondrocytes, which is evidenced by downregulation of the above chondrogenic markers genes and decreased proteoglycan synthesis.

Interestingly, we find that EPO/EPOR mRNA and protein 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.

Title(S3):The Research of Stem Cell Secretion on Differentiation of MSC

K.X. Wang and G. Li

Program: PhD; Supervisor: LI Gang

Mesenchymal stem cells in adult bone marrow are multipotent, they have an extensive proliferation capacity. Human bone marrow mesenchymal stem cells (hBMSCs) can differentiate into multiple lineages. MSCs synthesize a lot of growth factors and cytokines. Exosomes are the primary mediators of MSCs' paracrine effect, they help to reduce tissue injury and enhance tissue repair. Stem cell secretion can promote multiple proliferation and differentiation of hBMSC. We tested four types of stem cell secretions: adipose tissue stem cell secretion, dental pulp stem cell secretion, gingival stem cell secretion and umbilical cored stem cell secretion. Alizarin red S (ARS) staining showed the minimal effective concentration of 10ug/ml dental pulp stem cells secretion or 20ug/ml umbilical cord stem cells secretion could promote strong osteogenesis of hBMSCs after treated for 11 days. The in vivo osteogenesis study using rat calvarial defect model is in progress. Other differentiation induction effects of the stem cell secretions will be examined.

Title(S4): Function of the BRE gene in spermatogenesis

Y. Yao, K.K.H. Lee.

Program: PhD; Supervisor: LEE Ka Ho Kenneth

The brain and reproductive organ-expressed protein (BRE) gene encodes a highly conserved protein found in a number of species including human and rodents. It was named BRE because it has been found to be highly expressed in the brain, testis and ovary of rat. BRE is a major component of the BRCA1 A complex which is known to play a vital role in the recruitment of BRCA1 to DNA damage sites. In adult mouse testis, co-expression of BRE and Oct4 was detected in spermatogonia and primary spermatocyte. However, how BRE deletion affects spermatogenesis has not been elucidated. Abolishing BRE expression may be embryo lethal. In anticipation of this possibility, we used Cre/loxP technology to control exactly when BRE is knockout. A mouse model that carried a Tamoxifen-mediated deletion of *BRE* exon 3 was generated to elucidate the function of BRE in spermatogenesis. We confirmed that the exon 3 of BRE has been excised using PCR. We also showed that the expression of BRE is significantly down-regulated in testis of Tamoxifen-induced BRE knockout (KO) mice. In addition, we found that BRE-KO had a profound effect on the testis. In control mice, the spermatogonia co-expressed BRE and Oct-4 (white arrows) and mainly distributed on the basement membrane of the seminiferous tubules. In striking contrast, in BRE-KO testis the OCT-4^{+} spermatogonia were distributed as multilayer instead of monolayer. Thus, my study suggested an essential role of BRE during spermatogenesis.

Vascular and Metabolic Biology

Title of Poster Presentation	Name	Abstract No.
Effects of protease activated receptors on a rat model of brain ischaemia	X. Zhen	V1
Bone Moprhogenic Protein-4 Impairs the Endothelial Function through Increasing of Oxidative Stress in Type 2 Diabetic Mice	Y. Zhang	V2
Role of 5-Hydroxytrptamine-induced Reactive Oxygen Species (ROS) production in Human Umbilical Vein Endothelial Cells Contraction	Q. Zhang	V3
Nitric Oxide Inhibites 11,12-EET induced Porcine Coronary Arterial Hyperpolarization and Relaxtion	P. Zhang	V4
The Role of Lipocalin-2 in Vascular Dysfunction	L. Wang	V5
Upregulated TRPM2 and its potential role in neointimal hyperplasia	X.C. Ru	V6
Vitamin K2 and Vitamin D3 Supplementation Enhances Bone Matrix Mineralization via Calcium-sensing Receptor in Osteoblasts of Obese/Diabetic (db+/db+) Mice	C.C.W. Poon	V7
Endothelin-1 receptor inhibition improves endothelial function in Zucker fatty rats with metabolic syndrome	J. Liu	V8
Functional Role of TRPC5 Channels in Aortic Baroreceptor	E.O.C. Lau	V9
Oleic acid reverses palmitic acid-induced oxidative stress and impairment of endothelium-dependent relaxation	Z. Gao	V10
Peroxisome Profierator-Activated Receptor Delta (PPARδ) Mediates the Vascular Benefits of Metformin in Diet-Induced Obese Mice	W.S. Cheang	V11

Title(V1): Effects of protease activated receptors on a rat model of brain ischaemia

X. Zhen, E.S.K. Ng, F.F.Y. Lam

Program: PhD; Supervisor: LAM Fu Yuen Francis

Protease activated receptors (PARs) are a subfamily of seven-transmembrane G protein-coupled receptors. They can be activated through proteolytic cleavage of their N-termini to reveal new sequences that can bind to receptors to trigger signaling events. To date, four members of the PAR family have been identified, namely, PAR_{1-4}. $PAR_{1,}$ PAR_3, and PAR_4 are preferentially activated by thrombin, whereas, PAR_2 can be activated by trypsin and tryptase. PARs play surprisingly diverse roles and are implicated in a number of physiological and pathophysiological events. In this study, we aim to examine whether PARs have protective effects on a rat model of focal cerebral ischaemia induced by middle cerebral artery occlusion (MCAO). Activating peptides (AP) of PAR_{1-3}, but not PAR_4-AP, significantly improved symptoms of brain ischaemia when given at 30 min before the MCAO surgery. Thus, cerebral infarct, neurobehavioural deficit, and brain oedema were reduced. However, when they were administered at 15 min post-MCAO, only PAR_1-AP showed positive effects. Similarly, thrombin and trypsin improved the ischaemia symptoms when given prior to MCAO, but not after. The present findings suggest PAR_{1-3} could be potential targets for the development of new preventive drugs in the treatment of cerebral ischaemic diseases. PAR_1 is the most promising target as it showed remarkable effects when given before or after the MCAO-induced cerebral ischaemia. This indicates that PAR_1 could have both prophylactic and therapeutic potentials in the treatment of ischaemic brain diseases. The underlying mechanisms for the beneficial effects of PARs are currently being investigated.

Title(V2): Bone Moprhogenic Protein-4 Impairs the Endothelial Function through Increasing of Oxidative Stress in Type 2 Diabetic Mice

Y. Zhang, J. Liu, X.Y. Tian, W.T. Wong, Y. Huang

Program: PhD; Supervisor: HUANG Yu

Bone morphogenic protein (BMP4) stimulates superoxide production and exerts proinflammatory effects in the endothelium. BMP4 also mediates endothelial dysfunction in hypertension. However, the role of BMP4 in endothelial dysfunction of type 2 diabetes remains unknown. The present study aims to investigate whether inhibition of BMP4 can improve the endothelial function of type 2 diabetic mice, and to study the mechanism underlying BMP4-induced oxidative stress in diabetes.

db/db mice were infused with BMP4 inhibitor noggin (0.4 mg/kg/day) or vehicle through osmotic pump for two weeks, and vasoreactivities of mouse aortae and mesenteric arteries were measured. Isolated aortae of *db/db* mice were treated with BMP4 inhibitors including noggin, chordin, and follistatin for 24 hours. Reactive oxidative species (ROS) in both aortic endothelium and cultured endothelial cells were measured by DHE and CM-H_2DCFDA fluorescence.

Noggin infusion in *db/db* mice reduced systolic blood pressure without affecting the insulin and glucose tolerance. Endothelium-dependent relaxations (EDRs) in both aortae and resistance mesenteric arteries improved after noggin infusion in *db/db* mice. Treatment with noggin, chordin, or follistatin for 24 hours improved EDRs in *db/db* mouse aortae. ROS production was reduced by BMP4 inhibitors in *en face* endothelium of *db/db* mouse aorta. BMP4 treatment also increased ROS production in both *en face* endothelium of C57BL/6J mice and HUVECs, which was inhibited by BMP4 inhibitors, diphenyleneiodonium, and tempol. In addition, p38 inhibitor SB202190 or JNK inhibitor SP600125 also improved EDRs in *db/db* mouse aortae, and EDRs in C57BL/6J mice aortae impaired by BMP4.

The present study showed that inhibition of BMP4 *in vivo* reduces blood pressure and improves endothelial function of *db/db* mice. Oxidative stress, p38 and JNK activation contribute to BMP4-induced endothelial dysfunction in diabetes. BMP4 could be a potential therapeutic target in the treatment of diabetic vascular dysfunction.

Title(V3): Role of 5-Hydroxytrptamine-induced Reactive Oxygen Species (ROS) production in Human Umbilical Vein Endothelial Cells Contraction

Q. Zhang and Y.W. Kwan

Program: PhD; Supervisor: KWAN Yiu Wa

We have previously demonstrated that 5-hydroxytryptamine (5-HT, serotonin) metabolism (via MAO-A) caused reactive oxygen species (ROS) generation in human umbilical vein endothelial cells (HUVECs). In this study, experiments were designed to test the hypothesis that 5-HT-induced ROS production elicits contraction of HUVECs which may be important in the development of ECs damage during platelets aggregation.

Changes of the planar cell surface area (PCSA), myosin light chain phosphorylation (MLCP) expression and cell permeability (transendothelial electrical resistance (TER) and F-actin filament remodeling) were measured by electrical cell-substrate impedance sensing system and confocal microscopy, respectively. Protein expression of MLCP was determined using Western blot analysis.

In the presence of L-NAME (100 μM, 10 min), 5-HT (10 M, 30 min) consistently caused a significant reduction in PCSA and TER with an increase in pMLC expression. These effects were ameliorated by pre-treatment with PEG-catalase (500 Units/ml) or by gene knockdown of MAO-A procedures in HUVECs. In addition, 5-HT induced F-actin filament remodeling in HUVECs.

Our results demonstrate that exogenously applied 5-HT (in the presence of L-NAME to eradicate the influence of NO) consistently elicited contraction of HUVECs (probably via MAO-A mediated ROS generation) which resulted in the reduction of PCSA and TER, and increased MLCP expression as well as F-actin filament remodeling.

Title(V4): Nitric Oxide Inhibites 11,12-EET induced Porcine Coronary Arterial Hyperpolarization and Relaxtion

P. Zhang, Y. Ma and X.Q. Yao

Program: PhD; Supervisor: YAO Xiao Qiang

The vascular endothelial cells synthesize and release vasodilators such as Nitric oxide (NO) and epoxyeicosatrienoic acids (EETs). In normal physiological state, NO inhibits EETs-mediated vascular relaxation. In this study, we examined the molecular target of EETs and explored the mechanism of NO inhibition on EETs in isolated porcine small coronary arterial segments without endothelium.

Co-immunoprecipitation and immunohistochemistry studies demonstrated that TRPV4 (vanilloid transient receptor potential channel 4), TRPC1 (canonical transient receptor potential channel 1), and BK_{Ca} (large conductance Ca^{2+}-activated K^+ channels) physically interact with each other to form TRPV4-C1-BK_{Ca} tri-complex. Isometric force measurement and sharp microelectrode methods showed that 11,12-EET induced relaxation and membrane hyperpolarization in porcine small coronary artery, which were markedly reduced by treatments that inhibit the activity of TRPV4, TRPC1, or BK_{Ca}. 11,12-EET-induced relaxation and membrane hyperpolarization were inhibited by 8-Br-cGMP and NO donor S-nitroso-N-acetylpenicillamine (SNAP). This action was markedly reduced by treatments that suppress the protein kinase G phosphorylation on TRPC1.

This study uncovers a novel mechanism by which NO inhibits the action of EETs. We found that 11,12-EET acts on TRPV4-C1-BK_{Ca} complex to induce VSMC hyperpolarization and vascular relaxation in porcine small coronary artery, and that NO-cGMP-PKG inhibits 11,12-EET-induced membrane hyperpolarization by acting on TRPC1 component within the TRPV4-C1-BK_{Ca} complex.

Title(V5): The Role of Lipocalin-2 in Vascular Dysfunction

L. Wang, X.Y. Tian, W.T. Wong and Y. Huang

Program: PhD; Supervisor: HUANG Yu

Lipocalin-2 belongs to the lipocalin superfamily which is abundantly produced from adipocytes and strongly induced in inflammation, wound healing and pathogen invasion. Recent reports indicate the lipocalin-2 is associated with obesity, insulin resistance and vascular dysfunction. However, the mechanism is still unknown. This study aims to investigate the impact of lipocalin-2 in vascular function in diet- induced obese mice.

Lipocalin-2 knockout protected mice from developing the diet- induced vascular dysfunction. Organ culture of mouse aortae with lipocalin-2 impaired the endothelium-dependent relaxation and exaggerated the endothelium-dependent contraction in a concentration-dependent manner. The lipocalin-2 induced endothelial dysfunction was reversed by ROS scavenger and COX-2 specific inhibitor. Western blotting indicated lipocalin-2 induced BMP4 expression and decreased eNOS phosphorylation concentration-dependently.

Lipocalin-2, through activating the BMP4-COX2 pathway, is involved in the diet-induced endothelial dysfunction.

Title(V6): Upregulated TRPM2 and its potential role in neointimal hyperplasia

X.C. Ru, S. Wan, Y. Huang and X.Q. Yao

Program: PhD; Supervisor: YAO Xiao Qiang

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

Title(V7): Vitamin K2 and Vitamin D3 Supplementation Enhances Bone Matrix Mineralization via Calcium-sensing Receptor in Osteoblasts of Obese/Diabetic (db^+/db^+) Mice

C.C.W. Poon and Y.W. Kwan

Program: PhD; Supervisor: KWAN Yiu Wa

Hip fractures are commonly occurred in postmenopausal women especially with Type 1 and Type 2 diabetes mellitus (DM). During bone remodeling, extracellular Ca^{2+}-sensing receptor (CaSR) of osteoblasts (bone-building cells) plays an important role in sensing the changes in extracellular Ca^{2+} which are important for proliferation, differentiation and mineralization. Vitamins (K and D) are commonly prescribed to women for the improvement of bone microstructure. However, the role of CaSR especially under DM conditions, and the effects of vitamins K & D supplement on osteoblasts mineralization are not known. This study was therefore designed to evaluate the effects, in vitro, of vitamins K2 and D3 supplementation, alone or in combination, on bone mineralization and CaSR expression in osteoblasts of obese/diabetic (db^+/db^+) mice.

C57BL/KsJ (female; 6 months old) obese/diabetic (db^+/db^+) (leptin receptor-deficient) mice and its lean littermates/control (db^+/m^+) mice were used. Osteoblasts of both strains of mice were harvested from the iliac crests (both left and right), cultured and randomly assigned into control and drug-treated groups (vitamins K2 and D3 (1 and 10 nM), alone or in combination, incubated for 7, 14 and 21 days) for the determination of osteoblast mineralization (by measuring alkaline phosphatase (ALP) activity and Ca^{2+} deposits) and CaSR expression.

Osteoblasts harvested from obese/diabetic (db^+/db^+) mice, before drug treatment, have a significantly lower ALP activity, Ca^{2+} deposits and CaSR protein expression compared to lean (db+/m+) mice. Interestingly, vitamins K2 and D3 supplements increased ALP activities, Ca^{2+} deposits and CaSR expression in a concentration- and time-dependent manner, especially when both vitamins were applied in combination, with a greater magnitude of increase was observed in osteoblast of obese/diabetic (db^+/db^+) mice.

Our results clearly illustrate that osteogenic parameters (i.e. ALP activities, Ca^{2+} deposits and CaSR expression) measured are significantly lowered in isolated osteoblasts of obese/diabetic (db^+/db^+) mice compared to its lean littermates. More importantly, our novel results demonstrate that vitamins K_2 and D_3 supplements, especially given in combination, markedly enhanced bone matrix mineralization of osteoblasts of obese/diabetic (db^+/db^+) mice suggesting this drug combination strategy could be useful in treating osteoporosis especially under DM conditions.

Title(V8): Endothelin-1 receptor inhibition improves endothelial function in Zucker fatty rats with metabolic syndrome

J. Liu, X.Y. Tian, W.T. Wong, L.M. Liu, Y. Zhang, L. Wang and Y. Huang

Program: PhD; Supervisor: HUANG Yu

Cardiovascular disease is the leading cause of patients with metabolic syndrome and endothelin-1 (ET-1) is shown to be closely associated with cardiovascular disease. But whether ET-1 participates in the endothelial dysfunction in metabolic syndrome is not clear. The present work aims at investigating the role of ET-1 in vasculopathy in metabolic syndrome.

Six-month old Zucker lean rats and Zucker fatty rats were orally treated with or without ET-1 receptor antagonist bosentan for one month and then sacrificed. Renal arteries were dissected out and endothelium-dependent contractions (EDCs) were measured in wire myograph. Some renal arteries were kept for western blotting to measure the expression of ETAR, p38 AMPK and COX-2.

EDCs in response to acetylcholine were augmented in Zucker fatty rats compared with Zucker lean rats and were suppressed by chronic treatment with bosentan. Overnight treatment with bosentan, ETAR antagonist ABT627, p38 MAPK inhibitor SB203580 and COX-2 inhibitor NS398 also reduced EDCs in Zucker fatty rats. Moreover, overnight treatment with ET-1 induced EDCs in renal arteries from Zucker lean rats, which were inhibited by ABT627, SB203580 and NS398. Western blotting also showed increased phosphorylation of p38 MAPK and up-regulation of COX-2 after ET-1 treatment.

The present study showed an important role of ET-1 in the endothelial dysfunction in Zucker fatty rats, and the effect of ET-1 is possibly mediated by ETAR/p38 MAPK/COX-2 signaling pathway.

Title(V9): Functional Role of TRPC5 Channels in Aortic Baroreceptor

E.O.C. Lau, B. Shen, C.O. Wong, Y. Huang, X. Yao

Program: PhD; Supervisor: YAO Xiao Qiang

Aortic baroreceptor is the mechanosensor to detect blood pressure in aortic arch. Upon changes in arterial blood pressure, the baroreceptor nerve terminal on the aortic arch adventitia will be activated, resulting in action potentials that propagate to the cardiovascular control centre in the brain. However, the molecular identity of the baroreceptor mechanosensors is not well understood.
TRP channels are a superfamily of non-selective cation channels that can be divided into seven subfamilies: TRPA, TRPC, TRPM, TRPML, TRPN, TRPP, and TRPV. Many TRP isoforms have been reported to be sensors for diverse source of external and/or internal stimuli. Recently, one of the isoforms, TRPC5, has been reported to be hypo-osmolarity and pressure sensitive.

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

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

Title(V10): Oleic acid reverses palmitic acid-induced oxidative stress and impairment of endothelium-dependent relaxation

Z. Gao, H.N. Zhang, C.W. Lau, Z.Y. Chen, Y. Huang

Program: PhD; Supervisor: HUANG Yu

Palmitic acid and oleic acid are the two major components in the total plasma fatty acids, both participate in the regulation of vascular function. The present study examines the hypothesis that oleic acid protects against palmitic acid induced endothelial dysfunction collectively through reducing reactive oxygen species (ROS) production, down-regulating the COX-2 expression, and increasing nitric oxide bioavailability. The changes in endothelium-dependent relaxation of WKY rat carotid arteries were determined in wire myograph. The expression and activation of eNOS and COX-2 were analysed by Western blotting while ROS levels were measured by DHE fluorescence. Gas chromatography and mass spectrometry was applied to evaluate the levels of palmitic acid and oleic acid in Zucker fatty rats (an animal model of metabolic syndrome). The plasma levels of both palmitic acid and oleic acid are increased in Zucker fatty rats compared with lean rats. Overnight ex vivo treatment with palmitic acid impairs endothelium-dependent relaxations in WKY rat carotid arteries and elevates ROS accumulation across the vascular wall. Palmitic acid also reduces the phosphorylation of eNOS and increases the COX-2 expression in primary culture of rat aortic endothelial cells. All these harmful effects of palmitic acid can be reversed by the co-treatment with oleic acid. The present results suggest that palmitic acid impairs vascular function probably through stimulating ROS production and COX-2 expression in endothelial cells, leading to reduced eNOS activity and nitric oxide bioavailability, while oleic acid reverses these effects, indicating a functional importance of the regulation of different fatty acid species in mediating endothelial dysfunction in metabolic syndrome.

Title(V11): Peroxisome Profierator-Activated Receptor Delta (PPARδ) Mediates the Vascular Benefits of Metformin in Diet-Induced Obese Mice

W.S. Cheang, X.Y. Tian, W.T. Wong, C.W. Lau, Y. Lu, X. Yao, S.S.T. Lee, Y. Huang

Program: PhD; Supervisor: HUANG Yu

Metformin, an anti-diabetic drug, is known to activate AMP-activated protein kinase (AMPK); whilst AMPK and PPARδ have been shown to form a transcriptional complex and synergistically induce gene expression. The present study investigated whether PPARδ is a critical mediator for metformin in ameliorating endothelial dysfunction in diet-induced obese (DIO) mice.

Aortae from C57BL/6J mice were cultured with tunicamycin [endoplasmic reticulum (ER) stress inducer], metformin, or GSK0660 (PPARδ antagonist). Male *PPARδ* wild-type and knockout mice were fed with high-fat diet for 3 months to induce obesity, followed by oral administration with metformin for 7 days. Vascular reactivity and protein expression levels were determined by wire myograph and Western blotting, respectively. Fluorescence imaging was used to measure the levels of reactive oxygen species (ROS) and nitric oxide (NO) under confocal microscopy.

Tunicamycin impaired endothelium-dependent relaxations (EDR) in response to acetylcholine, and increased the levels of ROS and ER stress markers, such as phosphorylated eIF2α, ATF6 and ATF3 in mouse aortae. These harmful effects of tunicamycin were reversed by co-treatment with metformin while such benefits of metformin were abolished by GSK0660. Chronic metformin treatment alleviated EDR and reduced the levels of ROS and above-described ER stress markers in DIO *PPARδ* wild-type but not in *PPARδ* knockout mice. Tunicamycin-induced reduction of NO production in endothelial cells was also reversed by metformin.

The present study provides novel evidence that PPARδ plays a critical role in the vascular benefits of chronic metformin treatment in restoring the impaired endothelial function and curtailing ER and oxidative stress in obese mice. Dual medication of metformin together with PPARδ agonist could be a more effective venue against diabetic vasculopathy.

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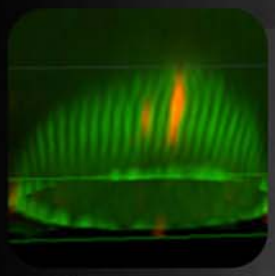

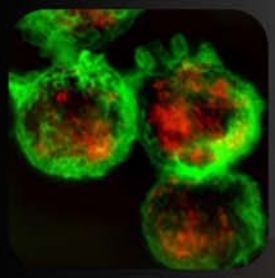

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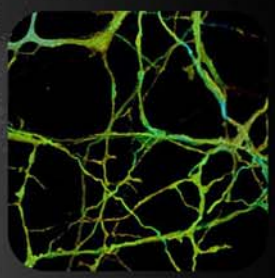

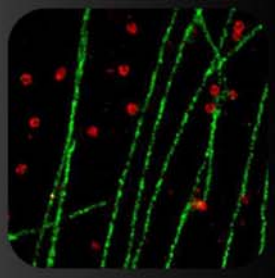

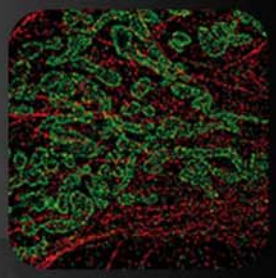

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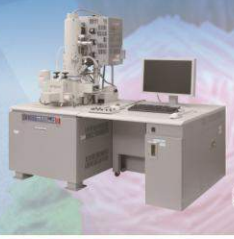

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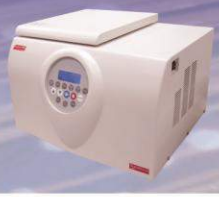

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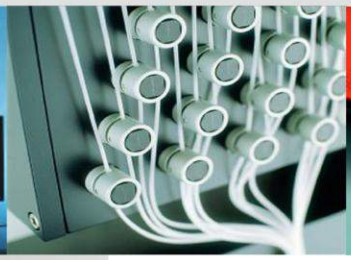



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