HONG KONG MEDICAL JOURNAL 香港醫學雜誌
SUPPLEMENT 1
VOLUME 15 ▪ NUMBER 1 ▪ FEBRUARY 2009

14th Medical Research Conference, 10 January 2009

Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Elucidating the genetic basis for human cancers

Plenary 1

ML Lung
Chair Professor of the Department of Biology, Director of the Center for Cancer Research, Hong Kong University of Science and Technology, Hong Kong

Cancers remain the number one cause of deaths in Hong Kong and many of the developed nations of the world. Although other major causes of death show a dramatic decrease since the last century, for cancer this is only beginning to decline. Earlier detection and improved treatment of cancers of local concern are important goals.

My laboratory is primarily interested in understanding the molecular genetic basis of two cancers of special concern to Chinese, namely nasopharyngeal carcinoma (NPC) and esophageal carcinoma (EC). These cancers occur predominantly among the Chinese. NPC is dubbed the "Guangdong tumour" because of its high incidence in the Southern Chinese. EC remains a major cause of deaths in parts of Northern China. We aim to better understand the genetic events that contribute to the development of these cancers, with the ultimate aim of providing new biomarkers for earlier cancer detection and prognostication, and for use as possible targets for novel therapeutics.

We have utilised several approaches, including monochromosome transfer, the gene inactivation test, and oligonucleotide and tissue microarrays, to identify candidate tumour suppressor genes that contribute to the development of these cancers. These studies have successfully localised critical regions associated with tumour suppression and have identified candidate genes involved in tumorigenesis. Our studies provide both functional evidence for their key roles in tumorigenicity and for determining their clinical significance. Several candidate tumour suppressor genes mapping to chromosome 3p were identified. Results of some of these studies will be discussed.

Research on gastrointestinal diseases in Asia: what lies beyond the horizon?

Plenary 2

FKL Chan
Professor of Medicine, Chief of Gastroenterology and Hepatology, The Chinese University of Hong Kong, Hong Kong

For decades, research on gastrointestinal diseases in Asia had been limited by the relatively narrow disease spectrum, inadequate resources of the region, and dominance of industry-driven studies. The true magnitude of many important clinical observations could not be evaluated because of a lack of large-scale, systematic data collection. Investigator-initiated clinical trials for assessing treatment strategies were uncommon. As a consequence, many Asian countries have adopted clinical practice recommendations from western countries. However, there is emerging evidence that some of these western guidelines may not be applicable in Asia. Recently, the outcomes of several landmark Asian studies not only changed the regional clinical practice but also rewrote certain recommendations in western countries. With increasing westernisation of gastrointestinal diseases in Asia, conditions such as gastroesophageal reflux disease, non-steroidal anti-inflammatory drug–induced gastrointestinal toxicity, and *H pylori*–negative idiopathic ulcers are increasingly recognised. Gastrointestinal research in Asia is likely to make an impact on global clinical practice. To meet this exciting challenge, we need to identify common goals on important research questions, establish multi-national systematic data collection, and train our fellows to conduct clinical trials according to international standards.

Is drug allergy more common in patients with systemic lupus erythematosus?

1

YL Au[1], MY Mok[1], Y Lo[1], WS Wong[1], P Wan[2], WS Chu[2]
[1]Department of Medicine, Queen Mary Hospital, Hong Kong
[2]Department of Family Medicine, Queen Mary Hospital, Hong Kong

Introduction: Patients with systemic lupus erythematosus (SLE) are more prone to infection. It is possible that these patients may be sensitised to antibiotics and develop drug allergy more frequently. This study aimed to compare the prevalence of drug allergy in SLE patients to healthy controls and to examine the association between past history of infective episodes and development of drug allergy in these patients.

Methods: A case-control study with 3:1 match design was performed comparing each SLE patient to three age- and sex-matched healthy controls. SLE patients (n=380) were recruited from rheumatology clinic whereas controls (n=1140) were recruited from staff clinic. Demographic data and history of drug allergy was retrieved from medical records and verified with subjects at clinic visits. Information on the number of major infective episodes that required commencement of antibiotics or hospitalisation was also recorded for SLE patients.

Results: The prevalence of drug allergy in SLE patients (26.3%, 100/380) was significantly higher than that of the controls (11.4%, 130/1140) [P<0.001]. Among these, antibiotic allergy was significantly more common in SLE patients compared to controls (17.6% vs 6.8%, P<0.001), particularly for sulphonamide (2.9% vs 1%) [P=0.01] and penicillin (11.8% vs 3.4%) [P<0.001]. There were no significant differences in the frequencies of allergy between SLE and control groups to ionic contrast medium, aspirin or non-steroidal anti-inflammatory drug. Those SLE patients with drug allergy (62/271) were more likely to have past history of major bacterial infection compared to those who did not have drug allergy (P=0.008).

Conclusion: Our study showed that SLE patients were more likely to develop drug allergy, especially to antibiotics and were found to be associated with past history of major bacterial infection.

Long-term prospective randomised study of hepatitis B vaccines without booster dose in children

2

DYK But[1], CL Lai[1], WL Lim[2], J Fung[1], DKH Wong[1], MF Yuen[1]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]Department of Health, Hong Kong

Introduction: Protective antibodies persisted up to 10 to 15 years after primary hepatitis B virus (HBV) vaccination. Titres of antibody against HBV surface antigen (anti-HBs) decrease with time. Necessity for routine booster dose remains controversial. Immunogenicity and efficacy of HBV vaccination without booster dose was examined in the present study.

Methods: From 1984 to 1986, 318 Chinese children from HBV families aged 3 months to 11 years old were randomised into one of the three HBV vaccine regimens without booster doses—group A: 2-dose recombinant; group B: 3-dose recombinant; group C: 3-dose plasma-derived vaccines. HBV surface antigen (HBsAg) titres, anti-HBs, and antibody against HBV core antigen (anti-HBc), were measured yearly.

Results: After 22 years, no subject was found to be HBsAg positive. Geometric mean titre (GMT) of anti-HBs of group A subjects was significantly lower than that of group B and C subjects at year 1, 5, 10, and 15. No difference was observed in the GMTs between group B and C throughout the 22 years. At year 22, the proportion of subjects with anti-HBs ≥10 mIU/mL (the protective level) for groups A, B and C were 35.3%, 76.5%, and 52.4% respectively. The difference was statistically significant between groups A and B. A total of 72 subjects had ≥1 episodes of anamnestic response with rises in anti-HBs titres after the primary vaccination during the 22 years study period. Of these, eight anamnestic responses were mounted from subjects with the preceding anti-HBs titre <10 mIU/mL. Three subjects became positive for anti-HBc.

Conclusion: Protective anti-HBs persisted up to 22 years after HBV vaccination without the use of booster dose. The 3-dose regimens have a better long-term immunogenicity. However, the 2-dose recombinant HBV vaccine was not inferior to 3-dose vaccines in terms of protection against chronic HBV infection. Booster doses were not necessary, due to long-term immune memory.

In-patient dermatology consultations in a tertiary hospital—retrospective study

3

CY Chan, CK Yeung, HHL Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Hospital-based dermatology unit serves the unique role in managing dermatological emergencies, assisting diagnoses of underlying systemic diseases, and treating co-existing skin diseases for in-patients. The objective of this study was to analyse the spectrum of in-patient skin disorders and the pattern of referrals.

Methods: In-patient consultations in a 33-month period (January 2006 to September 2008) in Queen Mary Hospital, a university hospital in Hong Kong, were evaluated on provisional diagnoses, in association with systemic conditions and pattern of referrals.

Results: A total of 1644 consultations were retrieved. The most frequent diagnoses were: eczema (18.9%), drug eruption (13.4%), and mycosis (6.9%). The top 10 diagnoses accounted for most referrals (75.2%). A total of 205 cases (12.5%) were associated with underlying systemic conditions. Dermatological emergencies accounted for 5.2% of all referrals. Blistering dermatoses, drug eruption, and pustular psoriasis were most commonly encountered dermatological emergencies.

Conclusion: The spectrum of in-hospital skin disorders differed from that observed in the out-patient sector. Drug eruption is one of the leading causes of in-patient referrals. Prompt identification and management of dermatological emergencies and assistance in diagnoses of underlying systemic diseases remain great challenges to in-patient dermatology service.

Protection of cigarette smoke–induced up-regulation of neutrophil elastase by Chinese green tea in rat lung

4

KH Chan[1], SCH Chan[2], SP Ho[1], SC Yeung[1], DKY Shum[2], MSM Ip[1], RYK Man[3], JCW Mak[1,3]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Medicine Biochemistry, The University of Hong Kong, Hong Kong
[3]Department of Medicine Pharmacology, The University of Hong Kong, Hong Kong

Introduction: Chronic obstructive pulmonary disease (COPD) is a progressively destructive airway disease mainly caused by smoking. Protease/anti-protease imbalance is hypothesised in the pathogenesis of COPD. Many studies have shown that the activities of proteases overwhelmed anti-proteases, causing lung destruction. Neutrophil elastase (NE) is one such protease released by neutrophil degranulation. This study aimed to investigate whether cigarette smoke (CS) exposure would up-regulate NE in bronchial secretions and whether Chinese green tea consumption would control the NE level/activity in the secretions.

Methods: Sprague-Dawley rats were divided into four groups, ie sham air, 4% CS, 2% Lung Chen tea plus sham air or 4% CS. Exposure to sham air or 4% CS was performed for 1 hour/day for 56 days in ventilated smoking chambers. Rat lung tissues and bronchoalveolar lavage fluid (BALF) were obtained from rats sacrificed 24 hours after last CS exposure. Activity and amount of NE were determined by activity assay and ELISA analysis respectively.

Results: NE activity was higher in BALF than in lung homogenates. There was a significant increase for NE activity in both lung homogenates (29.37±6.26 nM and 1.29±1.22 nM for CS-exposed and sham-air rats respectively; $P<0.001$) and BALF (43.47±3.15 nM and undetectable level for CS-exposed and sham-air rats respectively; $P<0.001$). The total amount of NE protein was also increased in BALF after CS exposure. The elevated CS-induced NE activity was prevented by green tea consumption (6.34±5.00 nM and 26.33±1.39 nM for lung homogenates and BALF respectively; $P<0.001$).

Conclusion: These preliminary data suggest that Chinese green tea might have the ability to suppress CS-induced up-regulation of NE activity and protein in lung. Further studies will be needed to elucidate the mechanism by which green tea regulates bronchial NE level in lung injury.

Acknowledgement: This research was supported by Hong Kong Lung Foundation Research Grant.

Expression of aquaporin-4 in thymus and thymoma of myasthenia gravis patients — a pilot study

5

KH Chan[1], DB Ramsden[2], SL Ho[1], PWL Ho[1], LC Cheng[3], WH Chui[3], A Sihoe[3], CW Ting[3], KHH Kwok[1], ACY Chu[1], JSC Kwan[1], MHW Kung[1]

[1]Division of Neurology, University Department of Medicine, The University of Hong Kong; Research Centre of Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong

[2]Department of Medicine, University of Birmingham, United Kingdom

[3]University Department of Surgery, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Neuromyelitis optica (NMO) is a serious central nervous system disorder characterised by monophasic or recurrent optic neuritis and acute transverse myelitis. 60 to 70% of NMO patients have detectable serum anti-aquaporin-4 (AQP4) antibodies. An autoimmune pathogenesis of NMO is suggested by clinical association of NMO with other autoimmune diseases especially myasthenia gravis (MG). Thymic abnormalities (hyperplasia and thymoma) are common in MG. The role of the thymus and thymoma in the pathogenesis of NMO is unknown. We aimed to study the expression of AQP4 in thymus and thymoma of MG patients.

Methods: Thymus and thymoma tissues were obtained from MG patients who underwent thymectomy as standardised treatment. AQP4 protein and mRNA in thymus and thymoma tissues were studied by western blot and reverse transcriptase PCR (RT-PCR). Positive control was human skeletal muscle (express AQP4); negative control was cultured human neuroblastoma cells (do not express AQP4).

Results: Four MG patients were studied. Thymoma (two encapsulated, one invading surrounding tissues) from three MG patients (two females; mean age, 37.3 years; range, 22-50 years) express AQP4 detected on western blot; one of the three had RT-PCR performed and was positive for AQP4 mRNA. A single female patient aged 28 years had MG associated with thymic follicular hyperplasia, whose thymus lysate was negative for APQ4 on western blot. All four MG patients were seropositive for anti-acetylcholine receptor antibodies, and none was seropositive for anti-AQP4 antibodies.

Conclusion: This pilot study revealed that thymomas of MG patients express AQP4 whereas hyperplastic thymus of MG patients does not express AQP4.

Anti-aquaporin-4 antibodies in idiopathic inflammatory demyelinating disorders

6

KH Chan[1], DB Ramsden[2], KHH Kwok[1], ACY Chu[1], PWL Ho[1], JSC Kwan[1], MHW Kung[1], SL Ho[1]

[1]Division of Neurology, University Department of Medicine, The University of Hong Kong; Research Centre of Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong

[2]Department of Medicine, University of Birmingham, United Kingdom

Introduction: Idiopathic inflammatory demyelinating disorders (IIDD) affect the central nervous system. In classical multiple sclerosis (CMS), brain, optic nerves (optic neuritis [ON]) and spinal cord (acute transverse myelitis [ATM]) are affected. In neuromyelitis optica (NMO), optic nerves and spinal cord are predominantly affected. NMO-IgG, an antibody targeting aquaporin-4 (AQP4) water channel, is a marker for NMO. We studied the frequency and clinical relevance of anti-AQP4 antibodies seropositivity in IIDD patients.

Methods: NMO-IgG was detected by indirect immunofluorescence using primate cerebellum.

Results: NMO-IgG was detected in 6 of 10 NMO patients (60%), 6 of 10 idiopathic relapsing transverse myelitis (IRTM) patients (60%), 2 of 9 idiopathic relapsing optic neuritis patients (22%), 1 of 11 patients (9%) having single ON attack, 1 of 30 CMS patients (3%), and none of patients having single ATM attack or controls. Comparing NMO-IgG–seropositive (n=12) with NMO-IgG–seronegative (n=8) patients having NMO or IRTM, NMO-IgG seropositivity was associated with a higher relapse rate in the first 2 years, 1.5 and 0.6 attacks/year for seropositive and seronegative groups respectively (P=0.006), and non-significant trend towards more severe ON and myelitis with poorer clinical outcome.

Conclusion: Anti-AQP4 antibodies facilitate diagnosis of NMO spectrum disorders and anti-AQP4 antibodies seropositivity is associated with higher relapse rate in the first 2 years.

Fractional carbon dioxide laser resurfacing for skin rejuvenation in Asians

7

NPY Chan, HHL Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Ablative fractional resurfacing (AFR) is a new modality for photorejuvenation, which combines carbon dioxide (CO_2) laser ablation with the concept of fractional photothermolysis. The objective of this study was to evaluate the efficacy and side-effects of a new fractional CO_2 ablative device (Fraxel Repair) for skin rejuvenation in Asians.

Methods: Each patient underwent one full-face treatment with the AFR device. The energy level ranged from 5 mJ to 60 mJ, with treatment level ranging from 7 to 9. Improvement in skin texture, skin laxity, wrinkles, enlarged pores, overall pigmentation irregularity, as well as post-treatment erythema, post-inflammatory hyperpigmentation and any adverse effects were noted up to 4 months post-treatment. Standardised photographs using the Canfield Visia CR system® were assessed by two independent observers. Subjective improvements were assessed by structured patient questionnaires.

Results: Six Chinese patients (skin type III-IV; mean age, 59.67 years) were included, five of which were treated for photoaging and the remaining one was for melasma. At last follow-up, statistically significant improvements were noted for skin laxity (P=0.042) and wrinkles (P= 0.039). Mild post-inflammatory hyperpigmentation and erythema were seen in 83.3% and 50% of patients respectively at last follow-up. Subjectively, all the patients were satisfied with the overall improvement.

Conclusion: Ablative fractional resurfacing with CO_2 laser is effective for the treatment of photoaging in Asians. Appropriate patient selection is important. Suitable candidates include males with photoaging or acne scarring and elderly females.

Safety study of transcutaneous focused ultrasound for non-invasive skin tightening in Asians

8

NPY Chan, SYN Shek, CS Yu, HHL Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The objective of this study was to determine the safety of a novel focused ultrasound device (Ulthera System) in the treatment of facial skin laxity in Asians.

Methods: The patients received one to two full-face treatments with the transcutaneous focused ultrasound device. Three transducers (7.5 MHz, 3.0 mm depth; 7.5 MHz, 4.5 mm depth; 4.0 MHz, 4.5 mm depth) were used to deliver a single pass of microthermal areas of coagulation without any topical anaesthetics. Standardised photos were taken with the Canfield Visia CR system® and all patients were clinically assessed for adverse effects up to 6 months post-treatment. Subjective assessments were also evaluated with patient questionnaires.

Results: Forty-nine Chinese patients (skin type III-IV; mean age, 53.3 years) completed a total of 58 treatment sessions. Focal bruising and numbness were present in up to 1.72% of treatment sessions. Two cases of mild post-inflammatory hyperpigmentation over the forehead were noted within 1 month post-treatment, both of which have responded to topical bleaching agent. The treatments were well-tolerated with an mean pain score of up to 6.85 out of 10.

Conclusions: Transcutaneous high-intensity focused ultrasound appears to be safe and well-tolerated for non-invasive facial skin tightening in Asians. Adverse events are mild and transient. No permanent or delayed side-effects were noted up to 6 months post-treatment.

9

Steatosis in chronic hepatitis B patients is associated with host metabolic factors and advanced fibrosis

P Chan[1], P Ip[2], T Yau[1], MF Yuen[1], DKH Wong[1], VWS Ngai[1], CL Lai[1]
[1]Department of Medicine, Queen Mary Hospital, Hong Kong
[2]Department of Pathology, Queen Mary Hospital, Hong Kong

Introduction: Steatosis of the liver has been described in patients suffering from hepatitis C, but its presence in chronic hepatitis B (CHB) has not been well-defined. The aim of this study was to determine the effect of steatosis in patients with CHB.

Methods: A case control study was conducted to review the effect of hepatic steatosis in treatment-naïve CHB patients. Hepatic steatosis and fibrosis were evaluated according to Brunt's and Knodell classifications respectively. The demographic, clinical, laboratory, and histological parameters of these patients were analysed.

Results: In 85 treatment-naïve CHB patients, 40 patients (47.1%) had steatosis. The median age was 41 years (range, 18-60 years). The majority of patients were male 65/85 (76.5%). Forty-seven (55.3%) patients were HBeAg-positive. The presence of steatosis was independently related to age (odds ratio [OR]=1.076, P=0.013) and abdominal obesity (OR=0.081, P=0.002). The presence of advanced hepatic fibrosis was independently related to steatosis (OR=0.180, P=0.003), Knodell necroinflammation activity (OR=0.208, P=0.037), decreased high-density lipoprotein level (OR=0.146, P=0.007), platelet count (OR=0.982, P=0.015), and prothrombin time (OR=1.535, P=0.002).

Conclusions: Steatosis in CHB infection is independently associated with the presence of advanced hepatic fibrosis. Host metabolic factors, namely age and abdominal obesity, are independent factors of the presence of steatosis.

10

Isoflavone reverses endothelial dysfunction, alleviates systemic inflammation, and enhances vascular repair leading to improved clinical outcomes in patients with cardiovascular disease

YH Chan, KK Lau, KH Yiu, SW Li, HT Chan, YT Fong, S Tam, CP Lau, HF Tse
Division of Cardiology, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Our prior study demonstrated that a higher intake of isoflavone was associated with enhanced vascular endothelial function and reduced carotid atherosclerosis in patients at high risk of cardiovascular events. This study aimed to investigate the effect of isoflavone on endothelial dysfunction, circulating endothelial progenitor cells (EPC), and clinical outcomes in patients with clinically manifest atherosclerosis.

Methods and Results:

Phase I study on endothelial dysfunction: A randomised controlled trial was performed to determine the effects of isoflavone (80 mg/day, n=50) versus placebo (n=52) for 12 weeks on the brachial flow-mediated dilatation (FMD) and highly sensitive C-reactive protein (hs-CRP) levels in patients with a history of ischaemic stroke. FMD at 12 weeks was significantly greater in isoflavone-treated patients (treatment effect, +1.0%; 95% CI, 0.1-2.0; P=0.035; relative increase, 51%). Isoflavone treatment was independently associated with significantly less impairment of FMD at 12 weeks (odds ratio=0.32; 95% CI, 0.13-0.80; P=0.014). In addition, isoflavone treatment for 12 weeks resulted in a significant decrease in serum hs-CRP (treatment effect, -1.7 mg/L; 95% CI, -3.3 to -0.1; P=0.033).

Phase II study on circulating EPC: A total of 102 consecutive patients (mean age, 66.5±9.5 years; 78% male; all female post-menopausal) with cardiovascular disease were studied. Flow cytometry was used to determine circulating levels of CD133+EPC. Isoflavone intake was positively associated with the level of circulating CD133+EPC (*r*=0.31, P=0.001). Higher intake of isoflavone from the first to the third tertile independently predicted an increase of circulating CD133+EPC level by 221 cells/μL (95% CI, 71.4-369.8; relative increase, 160%; P=0.004).

Phase III study on clinical outcomes: A total of 120 consecutive ischaemic/haemorrhagic stroke patients (mean age, 67±11 years; 70% male) were prospectively followed up for 30 months. By cox regression, higher isoflavone intake was an independent predictor for lower risk of stroke recurrence (HR=0.18; 95% CI, 0.03-0.95; risk reduction, 82%; P=0.043) and combined cardiovascular morbidities (HR=0.15; 95% CI, 0.03-0.78; risk reduction, 85%; P=0.024).

Conclusion: Isoflavone confers clinically significant secondary vasoprotective effects on both surrogate and clinical outcomes in patients with cardiovascular disease, on top of conventional risk factor interventions.

Heightened systemic oxidative stress critically accelerates worsening atherosclerosis in the late cardiovascular continuum

11

YH Chan, KK Lau, KH Yiu, SW Li, S Tam, CP Lau, HF Tse
Division of Cardiology, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Both increased oxidative and inflammatory stresses are implicated in atherogenesis. However, little is known about their role in atherosclerotic progression in patients already at the advanced cardiovascular continuum. This study aimed to investigate the impact of oxidative and inflammatory stress on the progression of carotid atherosclerosis in patients with established ischaemic stroke.

Methods: A total of 43 consecutive patients (mean age, 65.7±8.8 years; male, 70%) with primary or recurrent ischaemic stroke (>6 months) were recruited from our medical out-patient clinics. High-resolution ultrasound (Agilent Sonos 5500, Philips, US) was used to assess burden of carotid atherosclerosis in terms of maximum intima-media thickness (mIMT). Serum malondialdehyde (MDA) and high-sensitivity C-reactive protein (hsCRP) were respectively measured as markers of systemic oxidative and inflammatory stress.

Results: These patients showed a mean mIMT of 2.25±0.98 mm. Serum MDA (Pearson *r*=0.32, P=0.035) and hsCRP (Pearson r=0.41, P=0.007) were both positively associated with mIMT. Adjusting for potential confounders by multivariate model (age, gender, hypertension, diabetes mellitus, hyperlipidemia, smoking history, use of aspirin/statins/antihypertensives and body mass index), each 1 μM increase in serum MDA independently predicted increase in mIMT by 0.79 mm (95% CI, 0.23-1.36; P=0.008). Furthermore, each 1 mg/L increase of hsCRP was independently predictive of increase in mIMT by 0.06 mm (95% CI, 0.01-0.12; P=0.017). Hyperlipidaemia and diabetes accounted for IMT increase by 0.56 mm (95% CI, 0.04-1.08; P=0.037) and 0.53 mm (95% CI, 0.01-1.05; P=0.046) respectively.

Conclusions: This study demonstrated that systemic oxidative stress strongly accelerates secondary progression of carotid atherosclerosis in patients with established ischaemic stroke, independent of and above all conventional risk factors including systemic inflammation. This suggests that effective reduction of oxidative stress should be a major therapeutic target in patients at the advanced cardiovascular continuum.

An SLE lady with atypical neurological manifestation

12

SK Chang, W Mak, MY Mok
Department of Medicine, Queen Mary Hospital, Hong Kong

Introduction: Neuropsychiatric lupus comprises heterogenous manifestations and is often difficult to differentiate from other neurological diseases, such as stroke and infection.

Methods: To report a systemic lupus erythematosus (SLE) patient presented with atypical neurological manifestation.

Results: A 56-year-old woman with SLE and chronic rheumatic heart disease (CRHD) with mitral valve replacement presented with transient loss of consciousness. She had been stable on low-dose prednisolone, azathioprine and therapeutic dose of warfarin. On admission she was conscious but disordinetated, apathic and obtunded. There was no fever or neck rigidity to suggest sepsis in the central nervous system or clinical or serological features to suggest flare up of SLE. Her right pupil was fixed and dilated. Vertical gaze was impaired. She was suspected clinically to have neuropsychiatric lupus. Neuropsychometric test revealed disorientation, impaired attention and anterograde amnesia. Routine blood tests were unremarkable and INR was 2.9. Anti-DNA, C3/C4, CRP and ESR were comparable to her usual levels. Electrocardiogram showed sinus rhythm. Echocardiogram did not reveal source of thromboembolism. Brain MRI and MRA showed features suggestive of bilateral thalamic infarct but did not reveal white matter lesion that was typical for neuropyschiatric lupus. Cerebrospinal fluid (CSF) analysis did not show pleocytosis or abnormal CSF protein level. She was thus suspected to have acute ischaemic stroke involving a variant of the paramedian artery, that runs from a common trunk named Percheron's artery, which arises from ipsilateral P1 segment of the posterior circulating artery supplying both thalamus. The patient was continued with warfarin and strengthened on cardiovascular risk factors control.

Conclusion: Paramedian thrombosis is an uncommon manifestation of stroke and can be mistaken as neuropsychiatric lupus because of atypical neurological presentation.

13

APPL1 as a novel regulator of hepatic insulin sensitivity and glucose homeostasis: molecular mechanism and physiological relevance

KY Cheng[1], MA Iglesias[3], KSL Lam[1], Y Wang[2], G Sweeney[4], WD Zhu[1], CL Hoo[1], EW Kraegen[3], A Xu[1,2]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Pharmacology, The University of Hong Kong, Hong Kong
[3]Garvan Institute of Medical Research, Australia
[4]Department of Biology, York University, Canada

Introduction: Insulin inhibits hepatic glucose production through activation of Akt, which in turn suppresses gluconeogenic gene expression. Hepatic insulin resistance contributes to both fasting and fed hyperglycaemia in patients with type 2 diabetes. APPL1, an adaptor protein, is involved in insulin actions in muscle cells and adipocytes. However, the physiological functions of APPL1 remain elusive. The major objective of this study was to investigate the role of APPL1 in insulin-induced inhibitory effects on gluconeogenesis in mouse models, and to investigate the cellular mechanisms involved.

Methods: For the in-vitro study, primary rat hepatocytes were infected with adenovirus encoding full-length of APPL1 or APPL1-specific RNAi for 24 hours, followed by starvation for 24 hours, and then treated with 10 nM insulin for various time points. Total cell lysate was collected for both immunobloting and real-time PCR analysis. For the in-vivo study, male *db/db* diabetic or C57 wild-type mice were injected with the adenovirus encoding full-length APPL1 or APPL1-specific RNAi via tail vein, respectively. Basic metabolic parameters were measured, and insulin tolerance test and glucose tolerance test were performed.

Results and conclusions: In rat hepatocytes, the effects of insulin on Akt phosphorylation and suppression of gluconeogenesis were markedly inhibited by adenovirus-mediated knockdown of APPL1, but were significantly enhanced by APPL1 overexpression. In *db/db* diabetic mice, hepatic overexpression of APPL1 alleviated hyperglycaemia and glucose intolerance, and increased insulin sensitivity. These metabolic changes were associated with increased Akt activation, and decreased expression of the key gluconeogenic genes in the liver. Conversely, specific knockdown of APPL1 expression in liver decreased insulin-mediated activation of Akt and induced a modest insulin resistance in C57 mice. Taken together, these data suggest that APPL1 is a key signalling molecule mediating the hepatic actions of insulin in suppression of gluconeogenesis via enhancing Akt activation.

Acknowledgement: This work was supported by General Research Fund (HKU 779707M).

14

Demonstration of hematopoietic hierarchy in acute myeloid leukaemia based on engraftment of different FLT3 internal tandem duplication clones in a xenogeneic transplantation model

AMS Cheung, HCH Chow, R Liang, AYH Leung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Acute myeloid leukaemia (AML) is a heterogeneous disease arising from a putative leukaemia stem cells (LSC) population. The latter are rare and replicatively quiescent but they give rise to proliferative myeloblasts, the pathological hallmarks for this disease. The Fms-like tyrosine kinase 3 internal tandem duplication (*FLT3*/ITD), present in one third of AML patients, has been shown to occur at LSC. In some cases, more than one *FLT3*/ITD occurs, implicating multiple leukaemic clones. In this study, we examined if these clones were arranged in a hierarchical fashion, based on the NOD/SCID mouse transplantation model, a gold standard for the enumeration of normal and leukaemic stem cells.

Methods: Purified $CD34^+$ cells from bone marrow (BM) or peripheral blood (PB) samples of AML patients were prospectively collected and independently reviewed by hematopathologists. They were transplanted intravenously or intraosseously into NOD/SCID mice. Leukaemic engraftments were assessed 6 to 11 weeks after transplantation by morphology and flow cytometry. Genomic DNA was extracted from pre- and post-transplantation tissues and *FLT3*/ITD were detected by PCR and DNA sequencing.

Results: A total of 81 samples (BM=70, PB=11) from 56 patients were collected. Of 81 samples, 20 showed *FLT3*/ITD of which 11 were transplanted into animals. Leukaemic engraftment was detected in seven of 11 AML samples. In two cases, the $CD34^+$ cells carried a single *FLT3*/ITD, which persisted in the engrafting leukaemic cells, even upon serial transplantation. Three cases carried two or more *FLT3*/ITDs. In one case, both *FLT3*/ITD clones were significantly reduced upon transplantation. In two others, one *FLT3*/ITD clone engrafted preferentially over the other.

Conclusion: Multiple *FLT3*/ITDs clones occurred in AML and they had different NOD/SCID repopulating potential, supporting the proposition that at least in some cases, the leukaemic clones were arranged in a hierarchical fashion.

The *FTO* variants are associated with obesity in the Hong Kong Chinese population

15

CYY Cheung[1], AWK Tso[1], PC Sham[2], A Xu[1], CHY Fong[1], RL Wong[1], DCY Yeung[1], KL Ong[1], BMY Cheung[1], KSL Lam[1]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]Genome Research Centre, The University of Hong Kong, Hong Kong

Introduction: Several recent studies have independently detected the association of obesity with the fat mass and obesity-associated gene (*FTO*). This gene is located on chromosome 16q12.2 and it contains nine exons in humans. This project aimed to perform a case-control association study together with an 8-year prospective study with a view to explore the effect of the *FTO* variants on the development of obesity and type 2 diabetes in the Hong Kong Chinese population.

Methods: We investigated five previously reported *FTO* variants and 13 *FTO* HapMap phase II tagging single nucleotide polymorphisms (SNPs) within an ~51kb LD block of *FTO* in a case-control study, involving 250 obese and 424 control subjects. Two obesity-associated *FTO* variants (rs8050136 and rs16952522) were further examined in an 8-year prospective study involving 1516 subjects of the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) cohort.

Results: We have replicated the association of the five previously published SNPs and detected a newly reported SNP (rs16952522) associated with obesity in our case-control study. Both *FTO* variants rs8050136 (P=0.003) and rs16952522 (P=0.010) were also associated with waist circumference. Moreover, SNP rs8050136 showed significant association with high-density lipoprotein (P=0.040) and rs16952522 was associated with fasting insulin level (P=0.019) and triglyceride (P=0.010). However, no positive association with rs8050136 and rs16952522 could be detected with glycaemia, obesity progression, and other related traits in the 8-year prospective study.

Conclusion: These findings suggest that the *FTO* variants are significantly associated with obesity in the Hong Kong Chinese population. Further functional studies of *FTO* should be carried out to explore the role of this gene in the development of obesity and insulin resistance.

Impacts of oseltamivir use in the control of influenza A outbreak in old-age-home residents

16

KC Chiu[1], LW Chu[1,2], J Luk[1], A Choi[3]
[1]Department of Medicine and Geriatrics, Fung Yiu King Hospital, Hong Kong
[2]Department of Medicine, Queen Mary Hospital, Hong Kong
[3]Department of Nursing, Fung Yiu King Hospital, Hong Kong

Introduction: Influenza outbreaks in old-age homes cause significant morbidity and mortality. In the past few years, our Hong Kong West Geriatric Outreach Team and the Centre for Health Protection would perform prompt on-site assessment and intervention during reported influenza outbreaks, including infection control measures and chemoprophylaxis treatment, except for those with relative contra-indications. The objectives of present study were to describe a prompt response strategy in the control of influenza A outbreak in old-age homes and to evaluate the clinical and health services outcomes of residents after oseltamivir chemoprophylaxis treatment.

Methods: This was a prospective cohort study. All subjects aged 60 or above living in old-age homes of the Hong Kong West Cluster which had influenza outbreak during 1 April to 31 July 2007 were included. In the evaluation of the hospitalisation rates of all subjects, we compared the pre-intervention versus post-intervention hospitalisation rates. In the comparison of oseltamivir-treated versus non-treated subjects, the outcome measures included the attack rate of influenza-like illness (ILI), and hospitalisation and mortality rates.

Results: During the study period, there were four outbreaks. The mean age and influenza vaccination rate of the subjects in these four old-age homes were 83.9 years and 89.4% respectively. The overall ILI attack rate was 11.2% and the case fatality rate was 7.1%. A total of 178 residents received oseltamivir prophylaxis and 54 did not because of relative contra-indication. The number of ILI cases decreased dramatically after the intervention. The hospitalisation rates decreased from 6.0% to 2.5% for pre-intervention versus post-intervention periods respectively. Regarding the outcomes of those residents with and without oseltamivir chemoprophylaxis, there were no significant differences between the two groups in terms of the development of ILI (0% vs 3.4%, P=0.342), hospitalisation rate (0% vs 2.8%, P=0.589), and mortality (0% vs 0.6%, P=1.000).

Conclusion: Influenza A outbreaks occur in well-vaccinated old-age homes and increase both hospitalisation and mortality rates. Prompt chemoprophylaxis together with general infection control measures is effective in the control of the outbreaks in old-age homes of Hong Kong.

17

Macrophage infiltration of adipose tissues is associated with reduced adiponectin secretion and insulin resistance in overweight Chinese women

WS Chow[1], DCY Yeung[1,2], TC Pun[3], A Xu[1,2], AWK Tso[1,2], RLC Wong[1], S Tam[2,4], KSL Lam[1,2]
[1]Division of Endocrinology and Metabolism, Department of Medicine, The University of Hong Kong, Hong Kong
[2]Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Hong Kong
[3]Department of Obstetrics and Gynaecology, Queen Mary Hospital, Hong Kong
[4]Clinical Biochemistry Unit, Queen Mary Hospital, Hong Kong

Introduction: Recent studies suggest that macrophage infiltration and increased cytokine secretion from non-adipocytes in adipose tissue play an important role in the development of obesity-related diseases such as type 2 diabetes. We investigated the correlation of adipose tissue macrophage infiltration with adiponectin production and systemic insulin resistance in Chinese.

Methods: Fifty-one non-diabetic Chinese women undergoing elective abdominal surgery for benign gynaecological conditions were recruited. Anthropometric parameters, blood pressure, lipid profile, fasting glucose/insulin, serum adiponectin, and various pro-inflammatory proteins were measured. Abdominal subcutaneous and visceral adipose tissues were collected during operation to study macrophage infiltration and adiponectin release from incubated fat explants. CD68 was used for the identification of macrophages.

Results: Overweight subjects (n=21), defined as BMI ≥23.0 kg/m^2, had greater waist circumference (84.1±6.6 vs 71.3±5.0 cm, P<0.001), higher systolic and diastolic blood pressure (118.5±12.0 vs 105.7±11.0 mm Hg, P=0.001 and 75.6±9.7 vs 66.5±6.6 mm Hg, P<0.001 respectively), higher LDL cholesterol level (3.15±0.80 vs 2.58±0.58 mmol/L, P=0.006), and were more insulin-resistant (homeostasis model assessment, HOMA-IR 1.4 [1.1-2.0] vs 1.0 [0.7-1.5], P=0.021) compared to normal-weighed subjects (n=30). Although serum adiponectin levels showed no statistical difference between two groups (4.0 [2.8-8.1] vs 5.7 [3.8-8.2] mg/L, P=0.192), overweight subjects have lower adiponectin levels in the media of both visceral and subcutaneous explants (104.1 [53.8-148.3] vs 179.4 (137.2-231.2) μg/L, P=0.001 and 53.7 (31.9-73.9) vs 80.8 (56.8-115.4) μg/L, P=0.017 respectively), and had higher macrophage infiltration at both sites (CD68 mRNA expressions, fold change 1.36, P=0.005 and 1.64, P<0.001 respectively). Partial correlation showed that adiponectin levels in serum and visceral, but not subcutaneous, explant medium, were inversely correlated with HOMA-IR (*r*= –0.505, P<0.001; *r*= –0.380, P<0.01; and *r*= –0.075, P=ns respectively).

Conclusions: This study showed that overweight subjects have higher macrophage infiltration in both visceral and subcutaneous adipose tissues, and reduced adiponectin production at these sites. Taken together, our findings suggest that even a modest increase in adiposity is associated with chronic inflammation of visceral adipose tissue, leading to systemic insulin resistance through the reduction in adiponectin production.

Acknowledgement: This work was supported by RGC7637/05M.

18

Eligibility of anti-tumour necrosis factor (TNF)–α treatment in patients with ankylosing spondylitis (AS) in Hong Kong according to the Assessment of SpondyloArthritis (ASAS) group recommendation

HY Chung, MY Mok, Y Lo, WS Wong
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Anti-TNF-α therapy has been shown to be efficacious in controlling ankylosing spondylitis (AS) that is known to be refractory to conventional disease-modifying drugs (DMARDs). The International Assessment of SpondyloArthritis (ASAS) working group has recommended criteria to select AS patients indicated for anti-TNF-α treatment. As this therapy is expensive, health care strategy in resource allocation is needed. This study aimed to evaluate eligibility for anti-TNF-α treatment in a Chinese cohort of spondylitis patients according to the ASAS recommendation.

Methods: Consecutive AS patients defined by the Modified New York criteria were recruited from the Rheumatology Clinic at Queen Mary Hospital. Medical records were reviewed for medications including DMARDs and non-steroidal anti-inflammatory drugs (NSAIDs). Disease activity was determined at clinic visit according to the Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). AS patients were considered eligible for anti-TNF-α therapy if BASDAI was ≥4 on a 0-10 scale and expert opinion based on clinical findings and failure of ≥2 NSAIDs in the past 3 months and failure of sulfasalazine for patients with peripheral arthritis.

Results: A total of 122 AS patients were recruited; 64 (52.5%) patients were found to have BASDAI >4 and 17 (13.9%) patients were found to have refractory disease according to expert opinion including failure of response to ≥2 NSAIDs. Assuming a prevalence of 0.7% for AS, 5850 among the 42 000 AS patients in Hong Kong were estimated to be eligible for anti-TNF-α therapy according to the ASAS guideline.

Conclusion: Of AS patients in our cohort, 13.9% has been found to be indicated for anti-TNF-α therapy giving an estimate of over 5000 patients needing this therapy in Hong Kong. This study provides information for health authority in health care planning in regard to allocation of resources.

Unravelling the regulatory genes of primitive hematopoiesis in zebrafish embryos based on microarray analysis of the chordin mutant

19

MIS Chung, CK Fung, HC Lin, AYH Leung
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: In zebrafish embryos, primitive hematopoiesis occurs in the intermediate cell mass (ICM) followed by definitive hematopoiesis arising from the ventral wall of dorsal aorta. We have previously reported the chordin mutant (Ch^M) in which primitive hematopoiesis was robustly expanded due to unopposed signalling of the bone morphogenetic protein. In this study, we attempted to identify genes with hitherto unknown functions which may play a role in the regulation of primitive hematopoiesis, based on gene expression profiling of the Ch^M.

Methods: A total of 498 expressed sequence tag (EST) were up-regulated in the tail region (where the ICM was located) of the Ch^M at 24 hours post-fertilisation (hpf). The ESTs were clustered according to build #113 of the zebrafish UniGene dataset. The gene name of each cluster was obtained either from the UniGene database or through sequence alignment with corresponding mouse and human sequences in the 'nr' database from NCBI using the tBlastx program. To prioritise functional screening of these genes, they were cross-referenced against the ZFIN whole mount in-situ hybridisation (ISH) database and the Mouse Genome Informatics knock-out mouse database. Specific gene function was retrieved from the Online Mendelian Inheritance in Man, Gene Ontology databases, and through literature searches.

Results: A total of 111 zebrafish newly reported genes were up-regulated in Ch^M. Together with the other 106 genes reported previously, a total of 217 genes were included in the analysis. Of 217 genes, 56 have known functions pertaining to hematopoiesis (41), vascular development (8), haemoglobin (5) and iron metabolism (2) and they were not further analysed. Twenty-two genes have been reported in the literature using zebrafish knockdown models but their roles in hematopoiesis have not been defined. The remaining 139 genes were associated with various cellular processes including apoptosis (7), cell adhesion and motility (12), cell cycle regulation (3), intermediary metabolism (18), signal transduction (11), transcriptional regulation (13), and other functions (56). Nineteen genes have no known cellular function. Experiments were in progress to characterise their expression in the zebrafish embryos and their functions in primitive hematopoiesis based on morpholino gene knock-down.

Conclusion: Microarray analysis of Ch^M has provided us with information about the novel genes which may play an important role in the regulation of primitive hematopoiesis in zebrafish embryos.

Zebrafish Jak2a/Stat5.1–mediated primitive hematopoiesis — when and how?

20

A Fan[1], ACH Ma[1], AC Ward[2], RS Lewis[3], R Liang[1], AYH Leung[1]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]School of Medicine, Deakin University, Geelong, Australia
[3]Walter and Eliza Hall Institute for Medical Research, Parkville, Australia

Introduction: The zebrafish Janus kinase 2a (jak2a) plays an important role in the initiation of primitive hematopoiesis at the hematopoietic stem cell (HSC) level but the temporal profile of jak2a signalling is not completely understood. It was investigated in this study with special reference to the effects of jak2a inhibition at different embryonic stage on hematopoietic lineage differentiation.

Methods: Expression of gene encoding for jak2a and stat5.1 was examined by in-situ hybridisation. The embryos were incubated with a soluble inhibitor of jak2a AG490 at various time points and the effects on primitive hematopoiesis were evaluated by in-situ hybridisation, flow cytometry. and quantitative real-time PCR.

Results: Zygotic jak2a and stat5.1 expression was first evident at 9 and 11 hours post-fertilisation (hpf). AG490 (50 μmol/L) treatment at 0-18 hpf significantly reduced gfp+ cells in dissociated Tg(gata1:gfp) embryos as well as the expression of genes associated with HSC (scl, lmo2), erythropoiesis (gata1, α-embryonic haemoglobin) and myelopoiesis (pu.1, mpo). Effects of AG490 could be rescued by injection of a constitutively active (ca) stat5.1 mRNA at 1-cell stage. AG490 treatment at 9-11 hpf reduced only erythropoiesis but not HSC or myelopoiesis. Intriguingly, AG490 treatment at 0-8 or 12-18 hpf had no effects on any of the lineages tested.

Conclusion: jak2a/stat5.1 initiates erythropoiesis at a critical time point between 9-11 hpf but its temporal profile of action on HSC and myelopoiesis would have to be further evaluated.

21

Task interference of prospective memory in healthy ageing and Alzheimer's disease: differential role of speed processing and response regulation

JL Gao, LW Chu, YS Chan, TMC Lee, RTF Cheung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Prospective memory (PM) is the memory for intentions in the future, and interference of these intentions on the present ongoing tasks is called PM task interference. Optimal PM would allow the elderly and patients with Alzheimer's disease (AD) to live independently. We conducted the present study to examine how healthy or pathological (ie AD) ageing would influence PM, and PM task interference.

Methods: We recruited 26 patients with mild AD, 40 age-matched healthy elderly, and 41 young subjects. We adapted the paradigm of Burgess (2003) to test event-based PM. To explore the underlying mechanism of possible PM deficit and increased PM task interference, we measured general processing speed by simple reaction time and response regulation by arrowhead test. The PM paradigm and simple reaction time were computer-based task programmed with E-prime™. The PM test had an ongoing block and a PM block. PM task interference was calculated by subtracting the pure ongoing task reaction time from that of ongoing task in PM block. Arrowhead task was a paper-based adapted test from Lee et al (2006).

Results: The PM task accuracy for the young, elderly, and AD patients were 82%, 87% and 69%, and the PM interference were 78 ms, 174 ms and 274 ms respectively. The accuracy of AD group was significantly less accurate than the other two groups ($P<0.05$), while no significant difference exists between the young and healthy old groups; the PM interference is bigger in the AD group than that in the health elderly group ($P<0.5$), which is bigger than that in the young group ($P<0.05$). Path analysis showed that it was the slow processing speed significantly mediated the ageing effect on increased task interference, and the deficiency in response regulation mediated the AD effect on increased task interference.

Conclusion: We conclude that the elderly is not impaired at event-based PM, while AD patients are. Further we find there is task interference of PM in all the three groups; this is line with the theory of Preparatory and Attentional and Memory processes (PAM model) in explaining PM interference. The double dissociation of the underlying mechanism lends support that the process of ageing and AD are similar in clinical feather but different in neuropsychological mechanism.

Acknowledgement: Supported by a grant from the Strategic Theme on Healthy Aging.

22

Effects of intermittent hypoxia and/or TNF-alpha on E- and A-FABP expression by human aortic endothelial cells in vitro

Q Han[1], SC Yeung[1], SP Ho[1], MSM Ip[1,2], JCW Mak[1-3]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Hong Kong
[3]Department of Pharmacology, The University of Hong Kong, Hong Kong

Introduction: Fatty acid-binding proteins (FABPs) are members of the lipid-binding proteins (LBPs) superfamily, regulating the fatty acid uptake and intercellular transport. Among nine subtypes identified with tissue-specific distribution, E- and A-FABP have been found to mediate atherosclerosis in animals. Obstructive sleep apnoea (OSA), characterised by recurrent intermittent hypoxia (IH) during nocturnal sleep, is closely associated with atherosclerosis. Circulating levels of proinflammatory cytokine TNF-alpha are increased in OSA. The objective of our study was to explore the effects of IH and/or TNF-alpha on E- and A-FABP expression by endothelial cells in vitro.

Methods: Human abdominal aortic endothelial cells (HAAEC) were exposed to intermittent normoxia (IN as control) or IH (a 5-min hypoxia [5% O_2] followed by a 10-min normoxia [21% O_2] for 64 cycles using the BioSpherix OxyCycler C42 system [BioSpherix, Redfield, NY]), in the absence or presence of TNF-alpha. The mRNA levels of E- and A-FABP in HAAEC were determined using RT-PCR.

Results: IH alone significantly up-regulated E- and A-FABP mRNA (1.25-fold and 1.44-fold vs control for E- and A-FABP respectively, $P<0.05$; $n=4$). The combination of IH and TNF-alpha caused additive effect on elevated mRNA expressions of E- and A-FABP (1.81-fold and 2.4-fold vs control for E- and A-FABP respectively, $P<0.05$; $n=4$).

Conclusion: We found that IH alone led to up-regulation, and to further enhancement of E- and A-FABP expression in presence of TNF-alpha by endothelial cells. Since elevated circulating TNF-alpha levels have been described in OSA, our data suggest that OSA may be prone to up-regulation of E- and A-FABP expression and thus atherosclerosis due to IH and inflammation.

Acknowledgement: This study was supported by HKU-CRCG Seeding Funding for Basic Research.

23

Identification and characterisation of the promoter region of human neuronal uncoupling protein-4, and its regulation to neuroprotection

JWM Ho[1], PWL Ho[1], KHH Kwok[1], MHW Kung[1], DB Ramsden[2], SL Ho[1]
[1]Division of Neurology, University Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Medicine, University of Birmingham, United Kingdom

Introduction: Uncoupling proteins (UCPs) are mitochondrial inner membrane proteins, which partially delink oxidative phosphorylation from ATP synthesis, and hence reduce reactive free radical formation. Among the five homologues, UCP4 is exclusively expressed in brain. We have shown that UCP4 is protective against MPP+ and dopamine-induced neuronal cell death, by suppressing free radical formation and maintaining intracellular ATP levels. However, the neuroprotection mechanisms and regulation of UCP4 are unknown. The aim of this study was to identify essential response elements involved in regulation of UCP4 expression.

Methods: 5′-RACE of human UCP4 transcripts were used to identify the transcription initiation site (TIS). A 3k-bp DNA fragment upstream of the TIS, and a series of 5′-deleted fragments (n=10) of the 5′-flanking region were cloned into pGL3-basic vector. The constructs were co-transfected with luciferase expression vector (pRL-TK) into SH-SY5Y and HEK293 cells. Cells were treated with HNE (NF-κB inhibitor) or cycloheximide (NF-κB activator) to study the effects of NF-κB signalling in mediating UCP4 gene expression by quantitative RT-PCR.

Results: Minimal promoter activity was observed within 100bp upstream of the transcription start site (+1). Two putative response elements, Sp-1 and CATT box, were identified at nt 55 and 25bp, respectively, upstream of the TIS. However, no TATA box was found. Deletion analysis showed that there was a significant increase in transcriptional activity in the presence of both Sp-1 and CATT box; lower but significant promoter activity was observed in the presence of either Sp-1 or CATT box. A NF-κB response element was identified at 515bp upstream of TIS. Activation of NF-κB by treatments of cycloheximide increased the UCP4 promoter activity, whereas inhibition by HNE resulted in significant suppression. The mRNA level of UCP4 was significantly modulated by NF-κB–mediated stimulation as shown by the quantitative RT-PCR.

Conclusions: Sp-1 and CATT box are important response elements that are sufficient to initiate gene transcription. NF-κB site might be crucial in the transcriptional regulation of UCP4 gene. The identification of NF-κB response elements in the promoter region, and its significant modulation of gene expression by NF-κB indicates an important link between the neuroprotective role of UCP4 and oxidative stress.

Acknowledgement: This research was supported by PhD Studentship Awards from the University of Hong Kong.

24

Role of neuronal uncoupling proteins in leptin-mediated neuroprotection against mitochondrial dysfunction in parkinsonian models

PWL Ho[1], JWM Ho[1], KHH Kwok[1], KH Chan[1], HF Liu[1], MHW Kung[1], DB Ramsden[2], SL Ho[1]
[1]Division of Neurology, University Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Medicine, University of Birmingham, United Kingdom

Introduction: Mitochondrial dysfunction, ATP deficiency, and oxidative stress cause neuronal cell death in Parkinson's disease (PD). Uncoupling proteins (UCP2, 4 and 5) are found in neurons. Partial uncoupling of ATP production from oxidative phosphorylation via UCPs results in less ATP but also less oxidative stress. Leptin is a hormone which regulates metabolic processes via signalling to the brain of the body energy store (fat). Leptin prevents neuronal cell death, but how it protects against mitochondrial dysfunction is unclear. We hypothesise that leptin is protective against mitochondrial dysfunction in neurons via UCP expression.

Methods: Knockdown of UCP2 expression was developed in human SH-SY5Y neuroblastoma cells by stable transfection of a microRNA expression vector against UCP2. Leptin (100 nM) were incubated under MPP^+-induced (0.5 mM) toxicity in both UCP2 knockdown and vector control cells for 24 hours. Neuroprotection by leptin was assessed by cell viability assay; ADP/ATP ratio, mitochondrial membrane potential and oxidative stress were measured by luciferase-luciferin bioassay and flow cytometry, respectively. The changes of UCP2, 4 and 5 expressions were determined by real-time RT-PCR and western blots.

Results: Leptin protected neuronal cell death against MPP^+-induced mitochondrial depolarisation and ATP deficiency, with an induction of UCP2 and UCP4 expression, but not UCP5. UCP2 knockdown abolished the neuroprotective effects of leptin. MPP^+-induced NADH accumulation and was potentiated by UCP2 knockdown. Treatment of leptin alone remarkably increased ATP level at 4 hours but declined to basal level after 48 hours.

Conclusions: Leptin increases UCP2 and UCP4 expression in our neuronal culture, and promotes neuronal survival by restoring MPP^+-induced mitochondrial depolarisation and ATP deficiency, which partly mediated by UCP2 expression. Our findings yield vital clues to regulators of neuronal UCPs, and to understand how leptin affects mitochondrial dysfunction in PD, and in other neurodegenerative disorders and ageing.

Acknowledgements: This research was supported by Research Grants Council, Hong Kong (HKU7661/07M; SLH), Donation Fund for Neurology Research (SLH) and the Henry G Leong Professorship in Neurology (SLH).

25

Methotrexate versus traditional Chinese medicine in psoriasis: a randomised placebo controlled trial to determine efficacy, safety, and quality of life issues

SGY Ho[1], CK Yeung[1], HC Liu[2], HHL Chan[1]
[1]Department of Medicine, Queen Mary Hospital, Hong Kong
[2]Clinical Centre for Teaching and Research in Chinese Medicine, Tung Wah Hospital, The University of Hong Kong, Hong Kong

Introduction: Psoriasis is a common and chronic immune-mediated skin disorder with no cure. To our knowledge, this is the first randomised placebo controlled trial comparing methotrexate and traditional Chinese medicine in terms of efficacy, safety, and quality of life issues for the treatment of psoriasis.

Methods: A total of 61 patients with moderate-to-severe plaque psoriasis were randomised to treatment with methotrexate, traditional Chinese medicine in a predetermined regimen or placebo for 6 months. The primary outcome was the psoriasis area and severity index (PASI). The secondary outcomes were the dermatologist global assessment (DGA), which highlights disease severity of socially visible sites, as well as the psoriasis disability index (PDI), which measures a patient's quality of life.

Results: Fifty patients completed the study and were included in the analysis. Dropout rates were highest in the traditional Chinese medicine group. Mean PASI change from baseline at 6 months was 73.9% in the methotrexate group, 15.1% in the traditional Chinese medicine group, and 32.0% in the placebo group. There was a statistically significant difference between the methotrexate, traditional Chinese medicine, and placebo groups, with methotrexate demonstrating greater effectiveness compared to both the other groups. No statistical difference was found when comparing the traditional Chinese medicine and placebo groups. The methotrexate group also showed greater improvement during assessments using the DGA and PDI.

Conclusion: Our results verify the therapeutic effect of methotrexate for the management of psoriasis. Despite widespread belief and use of traditional Chinese medicine in Asia for the treatment of psoriasis, this study failed to demonstrate efficacy of the current regimen.

26

Change in incidence, prevalence and characteristics of inflammatory bowel disease in a single centre in Hong Kong in the past three decades

SJ Hsu, FN Hung, PY Chan, YK But, OO Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The incidence and prevalence of inflammatory bowel disease (IBD) in the Asian population has remained low. However, a recent increase in both the incidence and prevalence has been observed.

Methods: We reviewed the clinical data of all the patients with IBD attending our Gastroenterology Clinic from 2000 to 2007 and compared to those in 1970-79 and 1980-89.

Results: A total of 37 Crohn's disease (CD) and 85 ulcerative colitis (UC) cases were analysed during the study period. The incidence of CD rose from 0.01 to 0.15 to 0.28 per 100 000 while UC rose from 0.10 to 0.14 to 0.64 per 100 000 during the periods of 1970-79, 1980-89, and 2000-2007. The prevalence of CD rises from 0.3 to 1.3 to 4.7 per 100 000 while UC rises from 1.1 to 1.5 to 10.7 per 100 000 during the three periods. The mean age of onset of CD and UC was 28±11 and 36±12 years respectively. The male-to-female ratio was 1.8:1 and 1:1. Smoking history was present in 13.5% of CD and 8.2% of UC. Family history of IBD was present in 2.7% of CD and 5.9% of UC. The mean duration of follow-up was 10.3±7 years for CD and 10±8 years for UC. A total of 48.6% of CD and 49.4% of UC has duration of disease for >10 years. According to the Montreal classification, 83.8% of CD patients presented at the age 17-40 years, and 16.2% >40 years. 27% has L1 disease, 21.6% L2, 51.3% L3, and 2.7% L4. 59.5% has B1 behaviour, 13.5% B2 and 27% B3, and 21.6% has peri-anal disease. Among the UC patients, 43.5% has E1 disease, 35.3% E2, and 21.2% E3. 35.1% of CD patients were in remission, 51.4% with mild-to-moderate disease, 8.1% with moderate-to-severe disease, and 5.4% with severe-to-fulminant disease. 88.2% of the UC patients were in remission, 4.7% had mild relapse of UC, 3.5% moderate, and 3.5% severe. Regarding colorectal cancer screening, only one patient (1/26) of UC was found to have moderate dysplasia while none has carcinoma identified.

Conclusion: IBD is on the rapid rising trend in Hong Kong but disease activity was usually mild and colorectal cancer seems to be a rare complication.

The study of *Helicobacter pylori* infection and methylation in non-neoplastic gastric mucosa

27

C Huang, AO Chan, MF Yuen
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Aberrant DNA methylation resulting in transcription silencing of cancer-related tumour suppressor genes is the earliest change in the gastric carcinogenesis. Our previous studies have confirmed that *Helicobacter pylori* infection induced gene methylation in non-neoplastic gastric mucosa. There is reason to believe that distinct patterns of epigenetic alterations may be discernible in apparently normal tissue as well as in cancers. This study aimed to profile epigenetic alterations induced by *H pylori* infection for early prediction of the risk of developing gastric cancer.

Methods: A genome-wide CpG methylation array was used to screen for gene methylation in normal gastric mucosa with *H pylori* infection. Samples without *H pylori* infection was used as a reference control. Methylation status of selected candidate genes was further confirmed by methylation-specific PCR (MS-PCR) and bisulphite sequencing. The effect of CpG island methylation on gene expression was analysed by RT-PCR.

Results: The differential CpG methylation array identified two groups of CpG island loci, which were hypermethylated in either *H pylori*–positive or –negative samples. Based on literature review, 10 genes (GSTP1, METAP2, PAX3, PIM3, CDH1, CTNNAL1, DLEC1, PTPN13, SNX9, and RIPK3) whose function might relate to *H pylori* status were selected for further validation by MS-PCR. Further experiments will be done to study the gene expression and its potential roles in cancer development.

Conclusion: The genome-wide screening of epigenetic alterations associated with *H pylori* infection revealed that there were some genes being hypermethylated. This may be one of the underlying pathways of *H pylori*–induced gastric cancer.

Impact of chronic diseases on quality of life measured by the SF-12v2 Health Survey — comparison with the population norm in Hong Kong

28

WW Huang, ETP Lam, CLK Lam
Family Medicine Unit, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Health-related quality of life (HRQOL) is a generic health outcome that allows direct comparison of the harmful effect of different diseases. Population norms enable the interpretation of significance of quality-of-life scores. The SF-12v2 is replacing the SF-36 as a HRQOL measure because it is much shorter but has similar sensitivity. This study aimed to establish the normative values of the SF-12v2 scale scores for the population and to determine the relative impact of different chronic diseases on quality of life.

Methods: A cross-sectional random telephone survey was conducted on 2410 Chinese adults randomly selected from the Hong Kong population with a structured questionnaire on socio-demographics, chronic morbidity and health service utilisation and the SF-12v2 Health Survey. The population mean scores for eight SF-12v2 domains were established. The Physical Component Summary (PCS) and Mental Component Summary (MCS) scores were calculated for each chronic disease group, which were compared with those without any chronic disease. The difference was tested by independent *t*-tests. The independent effects of each chronic disease on SF-12v2 PCS and MCS scores were tested by multivariate regressions.

Results: The mean PCS but not the MCS score decreased with age and females had lower scores than males. The total number of chronic diseases had a strong linear relationship with both PCS-12v2 and MCS-12v2 scores, with a reduction of PCS by 5 points and MCS by 2 points (out of a range of 100) for every chronic disease. Individual chronic diseases decreased PCS score by 2 to 5 points. The MCS score was adversely affected only by chronic lung or psychological diseases.

Conclusion: The population norm of the SF-12v2 Health Survey can be used as a 'normal' reference for the interpretation of scores from patients to assess the impact of their illnesses on their life. HRQOL should be included as a standard outcome measure for patients with chronic diseases.

Recurrence of hepatitis B–related hepatocellular carcinoma is associated with high viral load at the time of resection

29

IFN Hung, RTP Poon, CL Lai, J Fung, ST Fan, MF Yuen
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: To identify the risk factors for recurrence of hepatitis B virus (HBV)–related hepatocellular carcinoma (HCC) after resection.

Methods: A total of 72 patients who underwent liver resection for HBV-related HCC were recruited. Demographic, biochemical, tumour and viral factors at the time of resection were evaluated by univariate and multivariate analyses to identify risk factors associated with recurrence after resection.

Results: The median follow-up period was 18.9 months and the median age was 53 years, with male-to-female ratio of 59:13. Thirty patients developed tumour recurrence. Age >60 years, tumour size >5 cm, poorly differentiated tumour, lymphovascular permeation, the presence of microsatellite lesions, α-fetoprotein (AFP) level >1000 ng/mL and HBV viral load >2000 IU/mL (4 $\log_{10}$ copies/mL) at the time of tumour resection, HBV genotype C, core-promoter mutations and patients with no antiviral treatment after tumour resection were associated with increased cumulative risk of HCC recurrence. By multivariate analysis, HBV viral load >2000 IU/mL (4 $\log_{10}$ copies/mL) [P=0.001; odds ratio [OR]=22.3; 95% CI, 3.3-150.5), AFP >1000 ng/mL (P=0.02; OR=7.4; 95% CI, 2-26.9), tumour size >5 cm (P=0.02; OR=5.1; 95% CI, 1.3-19.8), and age >60 years (P=0.01; OR=4; 95% CI, 1.4-11.1) at the time of tumour resection remained to be the independent risk factors.

Conclusions: Viral load of >2000 IU/mL (4 $\log_{10}$ copies/mL) is the most important correctable risk factor for HCC recurrence after resection. Whether antiviral therapy in these patients can decrease tumour recurrence requires further investigations.

Does treatment with antidepressant drugs provoke violent behaviour?

30

CR Kumana, IJ Lauder
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: The assertion that initiation (or withdrawal) of antidepressant drugs, particularly selective serotonin reuptake inhibitors such as paroxetene, may precipitate violent and often self-harmful behaviour (including suicide),[1] is controversial. This confusion may have arisen as relevant prescription event monitoring was largely anecdotal, these types of drugs are actually prescribed for patients already at risk of such behaviour, and self-harm episodes are still relatively rare. Nevertheless, regulatory authorities in the UK urged caution regarding their use in young people. We therefore set out to evaluate the absolute hostility event rate associated with the initiation and withdrawal of paroxetene prescribed to patients for a variety of indications.

Methods: We calculated once-off number needed to treat (NNT) values for hostility events, based on available published data[1] of paroxetene treatment courses in adults and children for depression, obsessive compulsive disorder (OCD), anxiety, and premenstrual dysphoric disorder (PMDD).

Results:

Disease/disorder	Event rate		NNT (95% CI)
	Paroxetene	Placebo	
Depression	20/3799	8/2402	-517 (-190 to 720)
OCD	19/737	5/470	-66 (-33 to -8903)
Anxiety	16/3823	7/3404	-470 (-211 to 2112)
PMDD	5/760	0/379	-152 (-80 to -1401)
Overall	60/9219	20/6455	-238 (-164 to -430)*

* Weighted combined values

Discussion: Negative NNTs imply 'number needed to harm' (NNH), and smaller numbers indicate greater impact. The NNTs for OCD, PMDD and overall, are statistically significant and indicative of slight harm; the greatest effect being in OCD, though CIs are wide.

Conclusion: These findings suggest a slight overall tendency to harm (NNH=238), namely violent events (including self-harm) due to paroxetene therapy, but controversy persists.[2]

References

1. Healy D, Herxheimer A, Menkes DB. Antidepressants and violence: problems at the interface of medicine and law. PLoS Med 2006;3:e372.
2. Simon G. Antidepressants and suicide. BMJ 2008;336:515-6.

Adiponectin stimulates Wnt Inhibitory Factor–1 expression through epigenetic regulations involving the transcription factor specificity protein–1

31

JBB Lam[1,2,3], J Liu[1,2], KHM Chow[1,2], A Xu[1,2], RT Moon[4], KSL Lam[1], Y Wang[2]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Pharmacology, The University of Hong Kong, Hong Kong
[3]School of Biological Sciences, University of Auckland, Auckland, New Zealand; Howard Hughes Medical Institute, University of Washington School of Medicine, Seattle, US

Introduction: Adiponectin is a fat-derived hormone with multiple beneficial effects on obesity-related diseases. We have previously demonstrated that adiponectin is able to inhibit tumour development in mouse models of breast cancer and the growth of MDA-MB-231 human breast cancer cells in vitro through attenuation of the Wnt/beta-catenin signalling pathways. However, the detailed mechanisms underlying the inhibitory effects of adiponectin have yet to be fully elucidated. In this study we explored the potential involvement of Wnt Inhibitory Factor–1 (WIF-1), a Wnt antagonist frequently silenced in human breast cancer.

Methods and Results: Quantitative RT-PCR and western blotting showed that treatment with adiponectin increased WIF-1 mRNA level and its protein abundance in MDA-MB-231 cells. Similar to the effects of adiponectin, transient over-expression of WIF-1 significantly inhibited MDA-MB-231 cell growth in a time-dependent manner and inhibited nuclear beta-catenin activities, cyclin D1 expressions and serum-induced phosphorylations of Akt and glycogen kinase-3beta. Furthermore, studies using bisulfite genomic sequencing, methylation-specific PCR, and pyrosequencing revealed that adiponectin treatment decreased the number of methylated CpG islands located within the proximal region of the *Wif-1* promoter. Moreover, Sp-1 transcription factor binding sites were identified within the same region modulated by adiponectin treatment. Subsequently, western blotting demonstrated that adiponectin treatment also down-regulated Sp-1 expression.

Conclusions: Adiponectin elicits its inhibitory effects on MDA-MB-231 cell growth through its suppression of Sp-1 expression resulting in epigenetic-mediated up-regulation of the Wnt antagonist, WIF-1.

Acknowledgements: This project was supported by the grants from Seeding Funds for Basic Research of the University of Hong Kong (Y. Wang), Hong Kong Research Grant Council grant HKU 778007 (Y. Wang).

A high sequential organ failure assessment (SOFA) score predicted inferior outcome among haematology patients admitted to the adult intensive care unit because of infection

32

SS Lamb[1], SSY Wong[2], WM Chan[3], AYH Leung[1], A Lie[1]
[1]Department of Medicine, Queen Mary Hospital, Hong Kong
[2]Department of Microbiology, The University of Hong Kong, Hong Kong
[3]Adult Intensive Care Unit, Queen Mary Hospital, Hong Kong

Introduction: In this study, we examined the correlation between haematological, intensive care and microbiological indices and the outcome of patients with haematological malignancies who were admitted to the adult intensive care unit (ICU) because of infection.

Methods: All patients with haematological malignancies who were admitted to adult ICU of Queen Mary Hospital because of infection between July 2004 and June 2007 were retrospectively analysed. Univariate and multivariate analyses were performed to examine the clinical and microbiological factors which might impact on their mortality.

Results: There were 110 haematology patients admitted to ICU during the study period. Of these, 57 admissions due to infection were studied. The commonest causes of admission were bacteraemia (36.8%) and pneumonia (22.8%). 63.2% of patients had positive blood cultures upon ICU admission, of which *Escherichia coli* (29%) *Klebsiella pneumoniae* (10%), and *Stenotrophomonas maltophilia* (10%) were the commonest isolates. Mortality rates in the ICU (M_{ICU}), during that hospital admission (M_{HA}) and within 6 months of admission ($M_{6months}$) were 45.6%, 50.9% and 66.7% respectively. None of the following were found to have a statistically significant effect on the mortality rates: diagnosis, status of the underlying haematological malignancies, time from last chemotherapy, absolute neutrophil counts, antimicrobial treatment prior to ICU, or positive microbiological results. However patients with a high admission sequential organ failure assessment (SOFA) score >11 (SOFA provides scores for respiratory, cardiovascular, hepatic, coagulation, renal and neurological function) had a significantly higher $M_{6months}$ (94%) than those with a SOFA ≤11, $M_{6months}$ (51.7%). The SOFA score >11 has a sensitivity of 51.6%, a specificity of 93.3%, a positive predictive value of 94.1%, and a negative predictive value of 48.3% for $M_{6months}$. Of the 26 ICU deaths, 17 (81%) were in intubated patients versus 5 (56%) in non-intubated patients. Patients who required renal replacement in ICU invariably died (M_{ICU}: 100%).

Conclusion: A SOFA score >11 could identify haematology patients with an inferior outcome from ICU care. Its predictive value should be further evaluated in a prospective study.

33 Correlation between carotid intima-media thickness and virtual histology using IVUS in silent left main coronary artery disease

SWL Lee, MPH Chan, WKT Hau, SL Kong, KKW Chan, JSH Hai, SCC Lam, CWS Chan, HH Ho, LLT Lam, HF Tse, RHW Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Carotid intima-media thickness (cIMT) was used as a surrogate marker for prediction of atherosclerosis. Intravascular ultrasound (IVUS) and its derived modality, virtual histology (VH), were established as a complementary tool to coronary angiogram. The aim of the study was to examine any correlation between these two methods in patients with silent left main coronary disease (LMCD) requiring percutaneous intervention (PCI) to other coronary stenosis.

Methods: Patients with an angiographically 'normal' looking LM undergoing PCI to the LAD or LCx artery would received an IVUS examination. Silent LMCD was defined as 'normal' LM by angiogram and yet significant sub-intimal disease (luminal area loss >20%) by IVUS. Patients fulfilling these criteria were enrolled. cIMT parameters (bulb, internal and common arteries on both sides) were measured, using IE33 auto-edge detection program. IVUS-VH data were obtained by the Volcano system. Plaque burden (area obstruction in mm^2 at narrowest segment) was also measured. Measurements of either method were obtained in a blind fashion. Pearson correlation coefficient was used for statistical analysis and a P value of ≤0.05 was considered significant.

Results: Twelve patients with silent LM disease (mean age, 67.4±9.6 years; 8 men) were enrolled to date. cIMTs were within normal limits in all patients, with a mean of 0.65±0.11 mm. IVUS-measured mean LM luminal area was 13±3.75 mm^2 and mean plaque burden was significantly high at 51±6.9%, despite the 'normal'-looking LM by angiogram. The mean percentages of various VH morphologies, fibrous, fibro-fatty, necrotic core and calcium were 58.5±10.15%, 24.0±11.9%, 11.2±11.2% and 6.3±6.4%, respectively. No significant correlation was found between the cIMT and the plaque burden, nor the four types of VH morphology.

Conclusions: cIMT is not able to predict the presence of silent LM coronary disease as revealed by IVUS and VH. There is no correlation between cIMT and IVUS plaque burden and VH morphologies.

Acknowledgement: This research was supported by Pfizer Investigator-Initiated Research Grant.

34 Survival from cardiovascular events as predicated by carotid (common carotid artery) intima-media thickness and Doppler values in ischaemic Chinese patients requiring coronary angiogram

SWL Lee, SCC Lam, SL Kong, YM Lam, WKT Hau, JSH Hai, MPH Chan, KKW Chan, LLT Lam, CWS Chan, RTF Cheung, HF Tse, HW Chan
Cardiology, Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Carotid intima-media thickness (cIMT) of the common carotid artery may be correlated to the presence of coronary artery disease. However, the role of the cIMT and Doppler findings in predicting subsequent cardiovascular events has not been established. The aim of this study was to investigate the predictive value.

Methods: A total of 212 consecutive Chinese patients undergoing clinically driven coronary angiogram were evaluated. Carotid parameters (cIMT, peak systolic velocity [PSV] and end diastolic velocity, at the bulb, internal and common carotid artery, on both sides) were analysed using the IE33 auto-detection program. Patients were followed up and monitored for the occurrence of the primary composite end-point (PCEP), defined as all cardiovascular deaths, non-fatal MI, stroke, CHF, ACS or arrhythmias requiring hospitalisation or intervention.

Results: To date, the mean follow-up time was 534±234 days. The PCEP was reached in 25 subjects (11.8%). The right common cIMT was shown to be a predictor for critical coronary stenosis requiring PCI (area under ROC curve=0.626, P=0.001). cIMT <0.8 mm was associated with a better survival (log rank test, P=0.029). Univariate analysis, a right common cIMT of ≥0.8mm, the right common carotid PSV, and the waist circumference were independent predictors for the PCEP. Cox proportional hazards model adjusted for age showed the right common carotid PSV was the only independent predictor for the PCEP (HR=0.963; 95% CI, 0.939-0.987, P=0.002). Similar correlations were not observed with the overall (left and right) cIMT or the left cIMT in this study.

Conclusion: The right common carotid PSV and the right common cIMT ≥0.8 mm are shown to predict the cardiovascular survival in this study. The latter also strongly correlates with the presence of critical coronary stenosis requiring intervention.

Hyperproduction of interleukin-23 P19 in chronic hepatitis

35

J Li[1], HY Zhang[1], K Man[2], YC Chen[1], JM Luk[2], DY Guo[2], CC Ling[2], YH Yueng[1], GKK Lau[1]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Surgery, The University of Hong Kong, Hong Kong

Introduction: Interleukin-23 (IL-23) is an important cytokine in chronic disease, which is constituted by its specific subunit IL-23 p19 and IL-12/IL-23p40 subunit. Chronic hepatitis B (CHB) is an abnormal immune response to hepatitis B virus caused chronic liver injury. The role of IL-23 in CHB is unclear, so we investigated expression of IL-23 in CHB.

Methods: A total of 130 CHB patients (divided into five groups according to their hepatitis B surface and e antigen expression and serum alanine aminotransferases level) and six normal controls were enrolled for full blood donation. Three normal liver tissues and five CHB liver tissues were obtained from liver transplant. Total RNA was isolated from liver tissues and peripheral blood mononuclear cells (PBMC). The mRNA level of IL-23 p19 was semi-quantified with Q-PCR and protein level of IL-23 p19 was detected with immunohistochemistry (IHC) technique.

Results: Except group 5 (recovery group), group 1 to 4 CHB patients expressed higher IL-23 p19 mRNA in PBMC than normal control: 1.23±0.23 vs 0.64±0.37, P=0.098; 1.71±0.34 vs 0.64±0.37, P=0.002; 1.45±0.25 vs 0.64±0.37, P=0.02; 1.57±0.33 vs 0.64±0.37, P=0.009 respectively. IL-23 p19 mRNA expressions in liver tissues agreed with the results got from PBMC: CHB patients expressed significantly higher IL-23 p19 in liver than normal control (24.05±6.85 vs 2.14±2.17, P=0.007). The IHC results confirmed above mRNA results showing that on protein level, IL-23 p19 were highly expressed in CHB liver compared to normal control. Further analyses showed that IL-23 p19 not only expressed on classic IL-23 expression cells such as Kupffer cells but also on some hepatocytes.

Conclusion: IL-23 p19 are highly produced in CHB not only through expression by classic IL-23 expression cells but also by hepatocytes.

Acknowledgement: We appreciate surgery department liver transplant group of Queen Mary Hospital and Professor Lan group for their kindly help in this study. This research was supported by 973 program (2007CB512800) and RPG (HKU 7679/06M) to Prof G. K. K. Lau and CSY foundation.

Clinical characteristics of polymyalgia rheumatica in a Chinese cohort

36

WL Li[1], MY Mok[1], Y Lo[1], MH Leung[2], WS Wong[1]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]Department of Medicine, Queen Elizabeth Hospital, Hong Kong

Introduction: The diagnosis of polymyalgia rheumatica (PMR) is chiefly based on clinical features including shoulder or pelvic girdle pain and stiffness and elevated erythrocyte sedimentation rate (ESR). Rheumatic conditions like rheumatoid arthritis (RA) can mimic PMR whereas some PMR patients may evolve into RA subsequently. This study aimed to examine the clinical characteristics of PMR patients including symptoms at first manifestation, clinical course and response to treatment in a Chinese cohort and to assess the predictive value of anti-cyclic citrullinated peptide (anti-CCP2) antibodies to subsequent development into RA.

Methods: Patients diagnosed to have PMR (ICD code: 725) during 1997-2008 at two large regional hospitals were identified via the clinical data analysis and reporting system and their medical records were retrieved for case verification and data analysis. Clinical features of these patients were compared to those reported in a Caucasian series (Bird et al, 1979). Rheumatoid factor (RF) and anti-CCP2 antibodies were determined by nephelometry and enzyme-linked immunosorbent assay respectively.

Results: Medical records of 44 Chinese patients were available for analysis. 77.3% of patients (n=34) were >65 years of age at diagnosis (mean±SD, 75.8±9.6 years). The commonest feature at disease onset was elevated ESR >40 mm/hr (44/44, 100%) followed by bilateral shoulder pain or stiffness (42/44, 96%). These features were comparable to the Caucasian cohort (P=0.17 and P=0.31 for ESR and shoulder symptoms respectively). However, Chinese patients had significantly longer duration of symptoms before diagnosis (P<0.001), less bilateral upper arm tenderness (P<0.001), and less generalised stiffness (P=0.01). Most patients showed a quick response to low-dose prednisolone. Twelve (27.3%) patients evolved into RA after a mean±SD duration of 7.5±10.0 months from onset of PMR. RF and anti-CCP antibodies were positive in 60% and 66.7% respectively among those evolved in RA (n=12) compared to 9.4% and 6.7% respectively among those who did not (n=32) during the period observed.

Conclusion: Chinese patients with PMR presented with longer duration of symptoms before diagnosis, less upper arm tenderness, and less generalised stiffness compared to the Caucasian counterpart. RF and anti-CCP2 antibodies were more likely to be present in those who subsequently developed into RA.

Genetic analysis of Alzheimer's disease–associated genes — a perspective from abnormal cholesterol metabolism

37

Y Li[1], LW Chu[2], YQ Song[1]
[1]Department of Biochemistry, The University of Hong Kong, Hong Kong
[2]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The physiopathology of Alzheimer's disease (AD) is complex and multifactorial. Recently, cholesterol pathway genes are found to be associated with risk of AD. The apolipoprotein E (*APOE*), ATP-binding cassette, subfamily A, member 1 (*ABCA1*), and cholesterol 24S-hydroxylase (*CYP46A1*) genes are key factors in cholesterol transportation and metabolism pathways. Genetic associations of *APOE ABCA*1 and *CYP46A*1 with AD have been analysed by different groups and across multiple populations, although with controversial and inconclusive findings. This study aimed to clarify the association of *APOE ABCA*1 and *CYP46A*1 with AD in the Hong Kong AD data set.

Methods: Case-control association studies, bioinformatics data mining, and statistical analyses were performed.

Results: The *APOE* ε4 allele was significantly associated with AD. Significant associations of *ABCA*1 and *CYP46A*1 with AD were observed in both single gene analysis and haplotypic association. Functional analysis on *CYP46A*1 promoter polymorphisms showed that different promoter haplotypes contributed differently to reporter genes expression, which linked genetic difference with the underlying patho-physiological mechanism.

Conclusions: Genetic analyses and association studies provided evidence that cholesterol pathway genes (*APOE, CYP46A*1, and *ABCA*1) were significantly associated with AD in the Chinese population, and the effect was in combination with the *APOE* ε4 allele.

Acknowledgement: This research was supported by a seed grant from the University of Hong Kong.

Role of C-reactive protein in diabetic kidney disease: pathogenic

38

F Liu, HY Lan
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Diabetic kidney disease (DKD) is the largest single cause of end-stage renal disease and its mechanism is much more complex and not clear. Chronic low-grade inflammation and activation of the innate immune system are closely involved in the pathogenesis of diabetes and its microvascular complications including DKD. C-reactive protein (CRP) is an acute-phase reactant of inflammation. Therefore, in STZ-induced diabetic CRP-Tg and Wt mice, we examined whether CRP may promote the progression of kidney injury.

Methods: Diabetes was induced by intraperitoneal injection of 50 mg/kg STZ for 5 days in CRP-Tg and Wt mice. Four experimental groups were studied: (1) non-diabetic control CRP-Tg mice; (2) non-diabetic control CRP-Wt mice; (3) STZ-induced diabetic CRP-Tg mice; and (4) STZ-induced diabetic CRP-Wt mice. Mice were assessed every month for blood glucose and albuminuria. Serum creatinine (Scr), a marker of renal function, was determined from cardiac blood when animals were killed at 24 weeks after STZ injection. Renal expression of proinflammatory cytokines was evaluated by real-time polymerase chain reaction and immunohistochemistry staining.

Results: In diabetic CRP-Tg and Wt mice group, with elevated blood glucose, albuminuria and Scr increased. Macrophage accumulation, glomerular and tubular damage, renal fibrosis, and kidney expression of inflammatory cytokines such as TNF, IL-1 in diabetic mice group increased. Compared to diabetic CRP-Wt mice group, diabetic CRP-Tg group has more diffuse and severe alterations.

Conclusion: This study suggests that CRP in diabetes is generally believed to play a significant role in the development and progression of DKD.

Uncoupling protein-2 mediates neuronal survival by leptin against mitochondrial dysfunction and ATP deficiency

39

HF Liu, WL Ho, ZJ Zhou, SL Ho
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Uncoupling proteins (UCPs) are mitochondrial solute carriers located in inner mitochondrial membrane. Five homologies (UCP1 to UCP5) share a common feature in a tripartite structure with three repeats of about 100 amino acids. UCP2 is ubiquitously expressed, and its neuroprotective effects and uncoupling activities are proved. Leptin is cytokine secreted by the adipocytes, which can modulate UCPs expression in the periphery. Some work that leptin might protect neurons against MPP+ induced cell death associated with UCP2 in human cell line were already done. UCP2-/-mice model will be used to prove that UCP2 is critical in mediating neuronal survival by leptin against mitochondrial dysfunction.

Methods:

Primary DA cell culture: The brain from pregnant mice (C57/B6 and UCP2-/-) at gestation day 14.5 were dissected, the ventral mesencephala excised and primary cultures were cultured in neuro-basal medium. On the ninth day in-vitro (DIV) cells were treated with leptin (100 nM) with or without MPP+ (10μM).

Identification of tyrosine hydroxylase immunoreactive (TH+) neurons: After treatment cultured cells were sequentially incubated with anti-tyrosine hydroxylase (anti-TH) antibody (Chemicon, US). Total TH+ cell numbers were counted in 10 randomly selected fields (1.13 mm^2/field) at 100 times magnification with a Nikon inverted microscope.

MPTP IP injection: MPTP (35 mg/kg) were I.P. injected into both WT and UCP2-/- mice during 2 weeks.

Leptin CNS infusion: leptin (14 ng/kg/min) over 14 days of treatment would be performed by minipumps.

Results: UCP2 is critical in mediating neuronal survival by leptin against mitochondrial dysfunction.

Conclusion: This study showed that leptin protected against MPP+ induced cell death and associated with UCP2. However, the mechanism of leptin and UCP2 still need more work.

Acknowledgement: This research was supported by UDM Departmental Research Grant, University of Hong Kong.

Destabilisation of LKB1 by SirT1: a novel anti-ageing mechanism through switching lysine acetylation to ubiquitination

40

L Liu[1], Y Wang[2], KSL Lam[1], A Xu[1,2]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Deparment of Pharmacology, The University of Hong Kong, Hong Kong

Introduction: SirT1, a highly conserved NAD^+-dependent protein deacetylase in mammals, has been reported to regulate the lifespan and a series of important physiological processes including apoptosis, fat metabolism, glucose homeostasis. LKB1, a tumour suppressor protein kinase, is an energy sensor that regulates cellular metabolism. Previous findings in our laboratory showed that LKB1 is a downstream target of SirT1 and lysine residues on LKB1 are acetylated. The objectives of this study were to investigate the mechanism whereby SirT1 regulates LKB1 and its subsequent cellular functions.

Methods: Stable HEK293 cell lines overexpressing FLAG-tagged LKB1 or SirT1 were established to evaluate how SirT1 regulates LKB1 protein stability and post-translational modifications. The effect of SirT1 on LKB1-mediated cellular senescence and cell cycle arrest were analysed by flow cytometry. Mass spectrometry (MS) analysis and site-directed mutagenesis were employed for determination of precise lysine acetylation sites on LKB1.

Results: Western blot analysis showed that SirT1 interacts with LKB1 and causes LKB1 lysine deacetylation as well as proteasome-mediated protein degradation. Flow cytometry, ^{3}H-thymidine incorporation and beta-gal staining results demonstrated that SirT1 antagonises the effects of LKB1 on inhibition of cell proliferation and promotion of cellular senescence. Proteomic analysis has identified six acetylated lysine residues on LKB1. Mutagenesis and immunocytochemistry studies revealed that mutations of LKB1 on lysine 48, 62, 64 to arginine could abolish the deacetylation effects of SirT1 on LKB1 protein stability and subsequent cellular responses.

Conclusions: LKB1 is a downstream target of SirT1 and SirT1 regulates LKB1 protein stability through either switching LKB1 lysine acetylation to ubiquitination or inducing LKB1 translocation from nuclei to cytosol where ubiquitin machines exist. Lysine 48, 62, 64 are the three sites of LKB1 through which SirT1 regulates LKB1. Through destabilisation of LKB1, SirT1 antagonises the effects of LKB1 on cell proliferation and senescence. These findings provide a novel mechanism whereby SirT1 exerts its anti-ageing activities.

Acknowledgement: Collaborative Research Fund (HKU 2/07C to Xu A).

Identification of three novel splicing variants of livin in acute myeloid leukaemia

41

CC Lo, AMC Cheung, TK Fung, RHC Lin, ACH Ma, MSY Chung, HCH Chow, AYH Leung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Livin is a member of the inhibitor of apoptosis (IAP) gene family, containing a single BIR domain and a C-terminal RING domain. The Livin α and β forms have been reported, differing by 54 base-pairs due to alternative splicing. In this study, we examined the expression of livin in both leukaemia cell lines and primary bone marrow (BM) samples from normal donors and patients with acute myeloid leukaemia (AML) and have identified three additional isoforms.

Methods: Expression of livin was first examined in cell lines derived from AML patients (NB4, KG1, and ML2). Specific isoforms of livin were identified by cloning the PCR products using DH5α cells followed by bidirectional DNA sequencing. Isoform-specific PCR was developed and their expression was investigated in primary AML samples and BM mononuclear cells (MNC) from normal donors. Livin V5, and livin α and β were selected to be transfected into the 293T cell lines for functional analysis.

Results: Livin mRNA was expressed in all three leukaemia cell lines. The PCR products were TA-cloned and 37 colonies were evaluated (NB4=10, KG1=15, ML2=12). In addition to livin α and β, three splice variants, referred here in livin V4, livin V5, and livin V7, were identified. They were different from livin β by 7, 178, and 9 base-pairs from alternative splicing at 5′ end of intron 5 with predicted protein sequences of 257m 224, and 283 amino acids respectively. Livin V4 and V5 were predicted to have frameshift resulting in disruption of RING domain. Livin V4 was identified from KG1 and ML2, while livin V5 and V7 were identified exclusively in NB4 and ML2 respectively. Fifty-six AML BM samples (MNC=46, CD34=10) were prospectively collected. Livin α was expressed in 29 samples and livin V4, V5 and V7 were expressed in 33, 21, and 20 cases respectively. Among 10 normal BM samples, livin α and V7 were expressed in six and seven cases respectively. Livin V4 and V5 were not expressed in any of normal samples. Livin V5 was successfully transfected and was diffusely expressed in the whole cell, while livin α and livin β were localised to nucleus.

Conclusion: Livin was expressed in AML BM in a heterogeneous fashion. Three putative novel isoforms were identified. The functions of livin V5 and its prognostic implication will be further evaluated.

The role of survivin2 (sur2) in primitive hematopoiesis during zebrafish development

42

ACH Ma, MIS Chung, R Liang, AYH Leung
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Survivin is a member of the inhibitor of apoptosis (IAP) family and its role in embryonic development is not completely understood. In zebrafish, survivin undergoes gene duplication. Survivin1 (sur1) has been shown to mediate angiogenesis but not hematopoiesis. In this study, we examined survivin2 (sur2) with particular reference to its role in primitive hematopoiesis during zebrafish development.

Methods: Anti-sense morpholino (MO), targeting at intron1-exon2 junction of sur2, was injected into wild-type and transgenic Tg(gata1:gfp) [in which the erythroid cells were GFP^+] embryos at 1-4 cell stage (referred herewith $Sur2^{MO}$ embryos). The $Sur2^{MO}$ embryos were evaluated in terms of morphology, hsemoglobin-staining, flow cytometry, TUNEL assay, in-situ hybridisation and Q-RT-PCR.

Results: Sur2 was expressed predominantly in the intermediate cell mass (ICM, site of primitive hematopoiesis). MO targeting at intron1-exon2 junction of sur2 significantly reduced GFP+ (erythroid) cell population in transgenic Tg(gata1:gfp) embryos at 18 hours post-fertilisation (hpf) [wild-type: 4.49±0.15%; Sur2MO embryos: 2.22±0.12%, P=0.02]. Molecular targeting was confirmed by RT-PCR and MO specificity by successful sur2 mRNA rescue. sur2 MO also down-regulated genes associated with hematopoietic stem cells (scl, lmo2), erythroid (gata1, alpha- and beta-embryonic hemoglobins) as well as early (pu.1) and late (mpo, l-plastin) myelomonocytic lineages at 12 and 18 hpf. The hematopoietic defects were associated with an increase in caspase activity and could be ameliorated by a specific caspase inhibitor. There was robust increase in apoptosis in the hematopoietic cells at the ICM.

Conclusion: Sur2 plays an important role in maintaining hematopoietic stem and lineage-committed cells during zebrafish development, by virtue of its anti-apoptotic activity in a caspase-dependent and cell autonomous fashion.

The study of Jak2 V617F mutation in polycythemia vera with zebrafish model

43

ACH Ma[1], AC Ward[2], RS Lewis[3], R Liang[1], AYH Leung[1]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]School of Medicine, Deakin University, Geelong, Australia
[3]Walter and Eliza Hall Institute for Medical Research, Parkville, Australia

Introduction: Polycythemia vera (PV) is a myeloproliferative disease resulting from clonal proliferation of hematopoietic stem cells (HSC). In most PV patients, the substitution of valine by phenylalanine at amino acid 617 in the auto-regulatory JH2 domain (V617F) results in constitutive activation of Jak2 ($Jak2^{V617F}$), conferring proliferative and survival advantage to erythroid progenitors. In zebrafish, the gene encoding for Jak2 has undergone duplication and subsequent specialisation into *jak2a* and *jak2b*. Previous studies have demonstrated that *jak2a* plays an important role in zebrafish primitive hematopoiesis via the Jak-Stat pathway. In this study, we aimed to establish a zebrafish model of PV based on *jak2a* mutation.

Method: In this study, a zebrafish $jak2a^{V581F}$ mutant clone, corresponding to human $Jak2^{V617F}$, was generated by site-directed mutagenesis. The mutant clone was in-vitro transcribed into mRNA and injected into early one-cell stage zebrafish embryos. The effects on erythroid and stem cell populations were examined using flow cytometry and quantitative reverse-transcriptase PCR (Q-RT-PCR).

Results: Injection of $jak2a^{V581F}$ mRNA up to 200 pg was compatible with normal development but higher dosage was associated with embryonic mortality. The effects on erythropoiesis was enumerated by the GFP^+ population in dissociated Tg(gata1:GFP) embryos. There was a significant increase in GFP^+ cells, which could be ameliorated by concomitant knock-down of zebrafish stat5.1 using MO (control: 4.37±0.08%; $jak2a^{V581F}$: 5.71±0.07%; $jak2a^{V581F}$+stat5.1 MO: 4.66±0.13%; P<0.01). The latter per se had no effect on the basal level of erythropoiesis (4.15±0.03%, paired Student's *t* test: P=0.153). Phosphorylation of stat5 was increased in $jak2a^{V581F}$ mRNA injected embryos. Genes encoding for erythropoiesis (*gata1, embryonic hemoglobin* α and β) were significantly up-regulated. *spi1*, associated with early myeloid differentiation, was also modestly increased. Expression of genes associated with HSC (*lmo2, scl*) and late (*mpo*: granulocytic; *l-plastin*: macrophage) myeloid lineages was unperturbed.

Conclusion: A zebrafish equivalence of $Jak2^{V617F}$ activated the jak2a-stat pathway and induced an increase in erythropoiesis. It may provide us with a zebrafish model of human PV.

Effects of cigarette smoke exposure on adiponectin levels in rats

44

JCW Mak[1,2], KH Chan[1], A Xu[1,2], SP Ho[1], RYK Man[2], KSL Lam[1], MSM Ip[1]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Pharmacology, The University of Hong Kong, Hong Kong

Introduction: Adiponectin, a member of the adipokine family, is a 247-amino acid protein mainly secreted by adipocytes and plays an important role in lipid metabolism and glucose homeostasis. Unlike many adipokines, plasma levels of adiponectin are significantly decreased in the obese, patients with diabetes, hypertension, dislipidaemia, or ischaemic heart disease compared with healthy individuals. Recently, cigarette smoke (CS) has been found to cause a decline in human serum adiponectin levels. The current study was undertaken to investigate circulating adiponectin level over time in an in-vivo experimental rat model after CS exposure.

Methods: Sprague-Dawley rats were randomly divided into two groups, ie sham air and 4% CS for a period of 7, 28, and 56 days. Exposure to sham air or 4% CS was performed for 1 hour/day in the ventilated smoking chamber. At the end of each time-point 24 hours after last exposure of CS, the animals were sacrificed. Serum and bronchoalveolar lavage fluid (BALF) were collected and analysed for adiponectin using in-house ELISA kits.

Results: Serum adiponectin levels were significantly decreased after 28-day (2885±361 vs 4841±557 ng/mL; n=10) and after 56-day (2537±321 vs 3771±460 ng/mL) but not after 7-day CS exposure (4772±694 vs 5838±654 ng/mL). In BALF, CS-induced reduction in adiponectin levels was also observed in comparison to sham-air group after 56 days (6.5±2.9 vs 42.3±19.9 ng/mL; n=5). Adiponectin was present with higher concentration in serum than in BALF. CS group showed a lower body weight gain over time than did SA group despite similar body weight at baseline.

Conclusion: We revealed that smoking exposure for a limited period of time was associated with low adiponectin level. The present finding may provide evidence of the importance of a causal relationship between smoking and adiponectin concentrations. Further studies will be required to elucidate the mechanism of CS on the regulation of adiponectin in circulation and in lung.

Acknowledgement: This research was supported by Hong Kong Lung Foundation Research Grant.

Coronary atherosclerosis using CT coronary angiogram in patients with systemic sclerosis

45

MY Mok[1], SS Chiu[2], Y Lo[1], KF Mak[2], WS Wong[1], PL Khong[2]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]Department of Diagnostic Radiology, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Myocardial insufficiency is present in approximately 25% of patients with systemic sclerosis (SSc). Both microvascular and macrovascular factors are likely contributory to the underlying pathogenesis. This study aimed to examine the frequency of coronary atherosclerosis in a series of SSc patients by CT coronary angiogram (CTCA), a less invasive method than conventional coronary angiogram, and to correlate CT findings with conventional and non-conventional vascular risk factors.

Methods: Nineteen consecutive SSc patients (6 diffuse and 13 limited disease) with disease duration of >3 years were recruited. Coronary calcium score and contrast angiography were examined by CT scan. Conventional vascular risk factors and inflammatory markers were screened and correlated with CT finding.

Results: Six (31.6%) patients were found to have detectable coronary calcium (calcium score range, 13-2019). Coronary calcium was detected in one diffuse SSc patient but contrast angiography was not done due to interference from implantable cardiac device in-situ. The other five (27.8%) patients had limited SSc and were found to have coronary atherosclerotic plaques of variable severity. All patients were asymptomatic. Univariate analysis identified age (P=0.001), ESR >20 mm/1st hour (P=0.03), disease activity score (P=0.02), pulmonary arterial pressure >35 mm Hg (P=0.03) and absence of anti-Scl70 antibodies (P=0.003) to be associated with abnormal CT findings.

Conclusion: Coronary atherosclerosis is common in asymptomatic SSc patients. CTCA is a safe and convenient imaging technique. Disease activity–related factors and clinical subset defined by SSc-specific autoantibodies may be vascular risk factors for coronary atherosclerosis in these patients.

Endothelial dysfunction is associated with low level of circulating endothelial progenitor cells in patients with systemic sclerosis

46

MY Mok, KH Yiu, CY Wong, J Qiuwaxi, WH Lai, Y Lo, WS Wong, HF Tse
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The potential mechanistic role of circulating bone marrow–derived endothelial progenitor cells (EPCs) for vascular repair in scleroderma (SSc) remains unclear. This study aimed to examine endothelial dysfunction in SSc patients and to correlate findings with biochemical markers of endothelial injury, circulating EPC count, disease activity, and organ involvement.

Methods: Endothelial dependent and independent vasodilation responses were assessed by changes in flow mediated dilation (FMD%) and nitroglycerin challenge (NTG%) in the brachial artery respectively in SSc patients compared to age- and sex- matched controls. Serum levels of VEGF and sVCAM-1 were measured by ELISA. Enumeration of circulating EPCs, identified as CD133/VEGFR2+ cells, was performed by flow cytometry.

Results: Median FMD% (4.8% vs 7.8%, P<0.001) and NTG% (17.0% vs 21.4%, P=0.002) were found to be significantly lower in SSc patients (n=52) than controls (n=52), especially in patients with limited disease SSC (lSSc). Median circulating EPC count was significantly lower in lSSc patients (23.0/μL) compared to controls (73.0/μL) [P<0.001]. This was accompanied by higher level of sVCAM-1 in these patients compared to dSSc patients (P=0.01). Univariate analysis revealed significant association of circulating EPC count with disease activity score, abnormal forced vital capacity, disease duration, lSSc subset and sVCAM-1 level. Multivariate analysis revealed that disease duration was the only independent predictor for circulating EPC count (P=0.04).

Conclusion: Endothelial dysfunction was demonstrated in SSc especially in patients with lSSc compared with controls; and correlated with biochemical evidence of endothelial injury and depletion of circulating EPC. These findings suggest that depletion of circulating EPCs might contribute to deficient mechanism in vascular repair in SSc patients.

Immunosuppressive effects of human mesenchymal stem cells on systemic lupus erythematosus (SLE) T- and B-cells

47

YJ Nie, CS Lau, CF Chan, A Chan, MY Mok
Department of Medicine, The University of Hong Kong, Hong Kong

Objectives: Our study aimed to investigate the immunosuppressive effects of mesenchymal stem cells (MSCs) from normal healthy donors on T and B lymphocytes of patients with systemic lupus erythematosus (SLE).

Methods: MSCs isolated from bone marrow of healthy donors were allowed to interact with peripheral blood T or B cells taken from healthy donors or patients with SLE in the presence of foetal bovine serum (FBS) or pooled sera from SLE patients. Cellular proliferation, surface marker changes, cytokine synthesis by T cells, and immunoglobulin production by B cells were measured.

Results: Normal healthy MSCs were able to suppress T- and B-cell proliferation. T cells were polarised towards $CD4^+CD25^+$ T and $Foxp3^+$ T-regulatory cells. IFN-gamma and IL-10 production were up-regulated. With B cells, there was down-regulation of the expression of CD27, CD38 and decreased secretion of IgG and IgM. The immunosuppressive effects of MSCs on B cells were abolished in the presence of SLE serum with increased expression of CD27 and CD38.

Conclusions: MSCs from normal healthy subjects inhibit proliferation of T and B cells, polarise T cells into T-regulatory cells, modulate T-cell cytokine production, suppress B-cell activation and regulate antibody secretion. However, SLE sera may inhibit the regulatory functions of MSCs on B lymphocytes.

Defective mesenchymal stem cells from patients with systemic lupus erythematosus

48

YJ Nie, CS Lau, CF Chan, MY Mok
Department of Medicine, The University of Hong Kong, Hong Kong

Objectives: To compare the phenotypic characteristics, telomerase activity and immuno-modulatory effect of mesenchymal stem cells (MSCs) from patients with systemic lupus erythematosus (SLE) to that of controls.

Methods: Bone marrow aspirates were obtained from SLE patients and healthy donors. SLE was defined by the American College of Rheumatology criteria. Controls were recruited from healthy donors for bone marrow transplantation. The study was approved by the IRB of HKU/HKWC. Bone marrow–derived MSCs were cultured and ex-vivoly expanded in alpha-MEM. The growth curve was completed by counting cells daily, phenotypes was measured by flow cytometry, activity of telomerase was detected with TRAPeze telomerase detection kit, multi-differentiating ability into adipocytes and osteocytes were investigated with Oil Red O and Alizarin Red S staining respectively, and T-cell suppressive effects was analysed by ^3H incorporation.

Results: Thirteen SLE patients and 16 normal controls were recruited for study. MSCs from SLE patients required longer time to achieve confluence during passage suggesting slower proliferation rate than healthy donors. Confluence was not observed in these SLE MSCs after passage 4. Both MSCs from SLE patients and healthy control were positive for CD29, CD73, CD90 and CD105, negative for hematopoietic markers CD45 and CD106. Their differentiating ability into osteocytes and adipocytes were similar. SLE MSCs demonstrated similar suppressive effect on T-cell proliferation compared to normal MSCs. However, SLE MSCs expressed telomerase activity while normal MSCs were negative.

Conclusion: Both MSCs from patients with SLE and healthy control have identical immunophenotyping profile. SLE MSCs were defective in proliferating potential, and expressed telomerase activity, but have normal multi-differentiating ability and immune-suppressive function on T cells. Whether the abnormal characteristics were related to the change in microenvironment in SLE patients deserved further exploration.

Elevated plasma level of soluble F11 receptor in hypertension

49

KL Ong, RYH Leung, A Babinska, MO Salifu, YH Ehrlich, E Kornecki, LYF Wong, AWK Tso, SS Cherny, PC Sham, TH Lam, KSL Lam, BMY Cheung
Department of Medicine, The University of Hong Kong, Hong Kong

Objectives: The F11 receptor (F11R), also known as Junctional Adhesion Molecule A, plays a role in the development of hypertension in rat. Genetic variants in the human F11R gene were demonstrated to influence systolic blood pressure. In the present study, we investigated the relationship between F11R and hypertension by examining the levels of a circulating soluble form of F11R (sF11R) in hypertensive patients.

Methods: The sF11R was measured by enzyme-linked immunosorbent assay in the plasma of 152 hypertensive and 166 normotensive subjects. Hypertension was defined as blood pressure ≥140/90 mm Hg or taking anti-hypertensive medication.

Results: Plasma sF11R levels were significantly higher in hypertensive subjects than in normotensive subjects (median [interquartile] range): 162.8 (85.5-293.2) vs 116.5 (74.1-194.8) pg/mL, P=0.004), which remained significantly higher after adjusting for age, sex, body mass index and homeostasis model assessment of insulin resistance index (HOMA-IR) (log-transformed) [P=0.028]. In stepwise multiple logistic regression analysis, the sF11R level (log-transformed) [P=0.040], triglycerides (log-transformed) (P=0.024) and HOMA-IR (log-transformed) [P<0.001] were independently associated with hypertension. In stepwise multiple linear regression analysis, only hypertension (P=0.013) and fibrinogen level (P=0.027) were significant independent predictors of sF11R level.

Conclusion: These results further support a role of F11 receptor in human hypertension.

Acknowledgement: The Hong Kong Cardiovascular Risk Factor Prevalence Study-2 (CRISPS2) was supported by the Hong Kong Research Grants Council grant (#7229/01M) and the Sun Chieh Yeh Heart Foundation. The study of single nucleotide polymorphisms was supported by the Hong Kong Research Grants Council Grant (#7626/07M).

Generalisation in myasthenia gravis presenting with pure ocular symptoms

50

SYY Pang[1], SL Ho[2], W Mak[1], RTF Cheung[2], TS Cheng[1], CT Tse[1], SK Chang[1], KH Chan[2]
[1]Division of Neurology, University Department of Medicine, Queen Mary Hospital, Hong Kong
[2]Research Centre of Heart, Brain, Hormone and Healthy Aging, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Myasthenia gravis (MG) is an autoimmune disorder characterised by impaired skeletal muscle neuromuscular transmission mediated by anti-acetylcholine receptor autoantibodies (AChRAb) in the majority of patients. Factors that determine generalisation in MG patients who have clinically restricted extraocular involvement at onset are uncertain.

Methods: Records of MG patients followed up in our neurology clinic were reviewed. Patients who had MG clinically restricted to extraocular muscles on initial presentation were studied. Classification and outcome were assessed according to Myasthenia Gravis Foundation of America (MGFA) 2001 recommendations.

Results: A total of 31 patients presenting with pure ocular symptoms at clinical onset were studied. Twenty patients (65%) were female with a mean follow-up duration of 92 months (range, 5-480 months). Among them, seven (22%) subsequently developed generalised MG at a mean age of 53.9 years (range, 23-72 years). The mean disease duration at generalisation was 48 months (range, 3-121 months). Four of these seven patients had thymectomy performed; two had reactive lymphofollicular hyperplasia, one thymoma, and one thymic lymphoepithelial carcinoma. Four patients continued to require cholinesterase inhibitors and immunosuppressants (MGFA post-intervention status [PIS] MM-3); two on low-dose cholinesterase inhibitor only (MM-2) and one required no pharmacological treatment in the past year (MM-0). There was no difference in onset age, sex, duration of follow-up, acetylcholine receptor antibodies and striated muscle antibodies seropositivity rates, electrophysiological findings between patients who developed generalised MG and patients who did not. Patients who subsequently developed generalised MG had a higher frequency of thymoma (defined by CT scan or histology) than those who did not (43% vs 8%, P=0.029). As expected, patients who developed generalised MG were more likely to be treated with immunosuppressants (57% vs 29%, P=0.033) and thymectomy (57% vs 4%, P=0.007) than patients who did not.

Conclusion: Presence of thymoma in MG patients who had disease clinically restricted to extraocular muscles on initial presentation is associated with a higher risk of subsequent MG generalisation.

Neuroprotective effects of self-assembling peptide nanofiber scaffold in a hypertension rat model of intracerebral haemorrhage

51

YH Sang[1], YX Liang[2], RG Ellis-Behnke[2], KF So[2], RTF Cheung[1]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Anatomy, The University of Hong Kong, Hong Kong

Introduction: Intracerebral haemorrhage (ICH) has the highest mortality rate in all kinds of strokes. Haematoma growth is associated with high systolic blood pressure (SBP) and increased mortality. In this study, we hypothesised that treatment strategies by removing blood from the parenchyma and stopping haematoma growth might reduce brain injury and we tested the effects of a self-assembling peptide (SAP) in a renovascular hypertension (RVHT) rat model of ICH plus blood clot aspiration.

Method: RVHT was achieved by applying a silver clip onto the left renal artery of male Sprague-Dawley rats. At 4 to 6 weeks after operation, rats with SBP greater than 150 mm Hg were selected to undergo ICH. At 3.5 hours after ICH onset, manual aspiration was performed to remove blood clot. Following the aspiration, 1% SAP solution or saline was injected into the lesion. Haematoma volume was quantified using Image J software at 24 hours after ICH. At 3 days after ICH, perihematomal cell death was determined with TUNEL staining. Inducible nitric oxide synthase (iNOS)-immunoreactive (IR) cells were detected immunohistochemically.

Results: Our results showed SAP treatment prevented hematoma enlargement compared with aspiration-only group and saline control group rats. The number of TUNEL positive cells in the perihematoma regions was reduced in SAP-treatment group (79.0±20.4 cells/mm^2), as compared with the other groups (333.2±34 cells/mm^2 for ICH-only group, 386.7±21.0 cells/mm^2 for aspiration-only group, and 214.9±54.1cells/mm^2 for saline control group). At 3 days after ICH, iNOS-IR–positive cells were present in the ipsilateral hemisphere. SAP treatment reduced iNOS expression (43.3±32.7 cells/mm^2), as compared with saline control group (234.3±133.3 cells/mm^2) and aspiration-only group (307.5±70.8 cells/mm^2).

Conclusion: Blood clot aspiration plus SAP administration after ICH protects the brain against both mechanical and chemical injury factors, resulting in a reduction in iNOS expression and cell apoptosis.

Acknowledgement: This research was supported by a CRCG Grant, The University of Hong Kong.

Interim analysis of the natural history of chronic hepatitis C genotype 1 and 6

52

WK Seto, CL Lai, J Fung, J Young, J Yuen, D Wong, MF Yuen
Department of Medicine, Queen Mary Hospital, Hong Kong

Introduction: Chronic hepatitis C virus (HCV) genotype 6 is found exclusively in Hong Kong and Southeast Asia, and is the second most prevalent genotype in Hong Kong after genotype 1. However data of the natural history of this genotype is scarce.

Methods: We compared the natural history of 141 patients with HCV genotype 1 (median age, 50 years) and 80 patients with HCV genotype 6 (median age, 47.5 years). Baseline demographic data including gender ratio, route of transmission, liver biochemistry, serial alanine aminotransferase (ALT) levels and HCV-RNA levels, as well as the rate of HCV-related complications were analysed.

Results: 72.3% of genotype 1 patients were infected through blood transfusion, 8.5% from intravenous drug addiction, compared with 56.2% and 28.8% for genotype 6 patients ($P<0.05$). There were no differences in the baseline liver biochemistry in terms of ALT, albumin, bilirubin, alpha-fetoprotein (AFP) and HCV-RNA between both groups ($P>0.05$). Comparison of the proportion of normal and abnormal ALT levels within both groups showed no statistical difference ($P=0.215$). There was also no difference in the cumulative risk of development of cirrhotic complications and hepatocellular carcinoma between both groups ($P=0.468$).

Conclusion: Both HCV genotype 1 and 6 share a similar course of natural history.

Acknowledgements: This research was supported by UDM Departmental Research Grant, HKU.

53

Bipolar radiofrequency with suction and light sources for cellulite, circumference reduction and skin texture improvement

SYN Shek, CK Yeung, SGY Ho, CS Yu, NPY Chan, HH Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: The bipolar radiofrequency with suction and light source device was cleared for use by the Food and Drug Administration for temporary reduction of cellulite. It has a reshaping effect on thighs, buttocks, abdomen, and arms. This leads to temporary reduction in the circumference of the treated area. The purpose of this study was to evaluate its performance for temporary circumference reduction, as well as improvement of cellulite appearance.

Methods: Twenty patients were recruited for six treatment sessions at weekly intervals. Patients were treated in two areas. The defined areas were both thighs, abdomen, buttocks, both arms and fatty region at waist. Subjects' weight, photography, cellulite level, circumference measurements were taken before first, fourth, sixth treatment and at 4-week follow-up. Patient satisfaction was rated.

Results: Fourteen subjects completed the course of treatment. Twelve subjects treated their abdomen, nine fatty region at waist, four buttocks, and three thighs. The mean abdominal circumference went from 91.60 to 90.30 cm at the sixth treatment and 90.84 cm at 4-week follow-up. Both are statistically significant with a P value of 0.010 and 0.036 respectively. Decrease in waist circumference at the sixth treatment was also statistically significant with a P value of 0.05. Mean circumference went from 93.1 to 92.2 cm. Other measurements were not statistically significant. One case of blistering was recorded which led to post-inflammatory hyperpigmentation.

Conclusion: Temporary improvement in body contour can be achieved by a course of six treatments. Abdomen and waist are the two areas that had the best outcome.

54

Hair removal with Nd:YAG laser and pneumatic skin flattening in Asian skin patients

SYN Shek, CK Yeung, SGY Ho, CS Yu, NPY Chan, HH Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Laser therapy has become the gold standard for removal of unwanted hair. It is known to incur immediate pain, to an extent that some have difficulties enduring the treatment. Pneumatic skin flattening (PSF) reduces pain by the 'gate theory'. The objective of the study was to assess the reduction in pain by PSF and the efficacy due to increased tolerance influence in Asians.

Methods: Twelve adults were recruited for laser hair removal in the axillae for a non-blinded randomised study. Subjects received treatment with standard laser procedure and laser procedure with PSF, PSF side with higher energy by 10 to 20%. Pain was assessed post-treatment based on visual analogue scale. Digital photographs were taken before treatment, after 20 minutes and follow-up visit at 8 and 36 weeks. Adverse effects were assessed by the operator immediately and 20 minutes after treatment.

Results: The immediate pain score for PSF was 0.75 less than that of the non-PSF. At 20 minutes post-treatment, PSF score was 0.16 less than that of the non-PSF side. Nine of 12 subjects preferred treatment with PSF. PSF caused more adverse effects. Seven subjects had immediate purpura. No post-inflammatory hyperpigmentation was observed.

Conclusion: PSF decreases pain sensation during laser hair removal in Asian patients but causes mild purpura after treatment.

Clinical pulmonary infection score reflects oxidative stress and mortality in patients with pneumonia in intensive care unit

55

WC Sin, JC Ho, BYC Pang, KYG Tang, WM Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Results of recent trials on antioxidant supplementation yield different outcomes but most of them failed to show survival benefit. Trials with survival benefit emphasised on specific patient population selection and early administration of high-dose antioxidant. Pneumonia is a common disease in the intensive care unit (ICU) with significant mortality. Oxidative damage has been shown as part of the disease mechanism. We conducted this trial to investigate the relationship between clinical pulmonary infection score (CPIS) and antioxidant level in ICU patients with pneumonia to facilitate patient stratification in further trial of antioxidant supplementation.

Methods: Patients admitted to ICU suffering from community-acquired pneumonia, nosocomial pneumonia and pre-existing ICU patients developed ventilator-associated pneumonia were recruited. Blood sampling for erythrocyte antioxidant assay, namely catalase, superoxide dismutase and glutathione was performed on day 0 and day 3 of admission. Clinical scores for disease severity (APACHE II score, SOFA score and CPIS) were calculated on day 0 and day 3.

Results: A total of 31 patients were recruited. There was no significant correlation between change in erythrocyte antioxidant level and change of CPIS from day 0 to day 3. CPIS 8 point was able to differentiate patient with low erythrocyte catalase and high erythrocyte catalase level (0.50±0.24 U/gHb vs 0.33±0.11 U/gHb; 95% CI, 0.04-0.31; P=0.013). Mean CPIS scores of non-survivor at the time of ICU admission were significantly higher than those of the survivors (8±1 vs 6±2; P=0.01).

Conclusion: CPIS can be used as a bedside tool to identify patients with pneumonia at risk of antioxidant depletion and guide subsequent antioxidant therapy. It also prognosticates patients with poor outcome.

Atorvastatin induces the expression of soluble receptor for advanced glycation end products

56

HL Tam, SWM Shiu, WS Chow, Y Wong, KCB Tan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Interfering with the activation of receptor for advanced glycation end products (RAGE) by using a soluble form of the receptor (sRAGE) prevents or ameliorates the vascular complications of diabetes in experimental studies. We investigated the effect of statin on the expression of sRAGE in vitro and in vivo.

Methods: THP1 cells were incubated with atorvastatin and sRAGE and esRAGE (a splice variant of sRAGE) was measured. The effect of atorvastatin on sRAGE and esRAGE was evaluated in 80 type 2 diabetic patients randomised to treatment with atorvastatin or placebo for 6 months.

Results: sRAGE and esRAGE were induced by atorvastatin in a time- and dose-dependent manner in THP1 cells. There was a significant increase in serum sRAGE and esRAGE in the atorvastatin group at 6 months ($P<0.05$) but no change in placebo group. Serum esRAGE was significantly higher in the atorvastatin group than the placebo group at 6 months ($P<0.01$) whereas the differences in sRAGE between the two groups did not reach statistical significance.

Conclusion: Atorvastatin can significantly increase the in-vitro and in-vivo production of the soluble receptors of RAGE. Whether modulating circulating esRAGE has a beneficial effect on diabetic complications will be evaluated in long-term prospective studies.

Acknowledgement: This project was supported by RGC General Research Fund, HKU 775708M.

Acute bacterial meningitis in patients with nasopharyngeal carcinoma

57

CT Tse, SL Ho, RTF Cheung, W Mak, SK Chang, KH Chan
Division of Neurology, University Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Nasopharyngeal carcinoma (NPC) has a high prevalence in South-East Asia. It has been reported that acute bacterial meningitis (ABM) in patients with NPC had a higher mortality rate than patients without NPC.[1,2] We aimed to study the clinical, radiological and cerebrospinal fluid (CSF) characteristics of ABM in patients with and without NPC.

Methods: Retrospective review of medical records of ABM patients admitted to neurology ward, Queen Mary Hospital, from January 1997 to June 2008 was conducted. Clinical, radiological and CSF characteristics were studied.

Results: A total of 26 ABM patients were managed during the study period. Mean age of the patients was 52.2 years, with male-to-female ratio 1.2:1. Positive CSF culture was present in 42% of patients. Five of the 26 had a history of NPC treated with irradiation prior to development of ABM. The mean interval between diagnosis of NPC to onset of ABM symptoms was 8 years (range, 1-23 years). There was no significant difference in presenting neurological features including mean Glasgow Coma Scale (15 in NPC group vs 13.8 in non-NPC group) between patients with and without NPC. Although there was difference in time interval between hospital admission and initiation of meningitic antibiotics therapy (60.2 hours in NPC group vs 12.9 hours in non-NPC group), there was no difference in duration of hospitalisation (21.8 days in both groups) or clinical outcome upon discharge measured by mean modified Barthel Index (80 in NPC group vs 85.8 in non-NPC group) and mortality rate (20% in NPC group vs 9.5% in non-NPC group).

Conclusion: ABM in patients with NPC treated by irradiation does not have worse neurological prognosis than ABM in patients without NPC.

References

1. Tang LM, Chen ST, Ng SH. Bacterial meningitis in patients with nasopharyngeal carcinoma. QJM 1996;89:71-6.
2. Huang CR, Lu CH, Chien CC, Lee PY, Chang WN. High incidence of gram-negative bacillary infection and high mortality in adult patients with bacterial meningitis and nasopharyngeal carcinoma. Eur J Clin Microbiol Infect Dis 2003;22:509-11.

Adipocyte–fatty acid binding protein as a biomarker for coronary atherosclerosis

58

AWK Tso[1], LHY Ong[1], A Xu[1,2,4], HKF Mak[3,4], CHY Fong[1], NMS Wat[1], PL Khong[3], KSL Lam[1,4]
[1]Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[2]Department of Pharmacology, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[3]Department of Radiology, The University of Hong Kong, Queen Mary Hospital, Hong Kong
[4]Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Obesity is associated with increased cardiovascular risk. Recent studies suggested that adipocyte–fatty acid binding protein (A-FABP) may have a role in mediating obesity-related metabolic and cardiovascular complications. Here, we investigated the association of baseline serum A-FABP with the severity of coronary atherosclerosis at 10 years in a Chinese cohort.

Methods: A total of 408 asymptomatic subjects without a history of coronary heart disease (CHD) at baseline underwent computed tomography for coronary artery calcium score assessment (CACS) at 10-year follow-up.

Results: Baseline A-FABP was significantly associated with the 10-year CACS ($P<0.00001$). A-FABP levels correlated with the Framingham risk score (FRS) at baseline ($r=0.48$, $P<0.001$). Significant additive effect of A-FABP to FRS in the prediction of coronary atherosclerosis at 10 years was observed, as demonstrated by significant likelihood ratios ($P<0.001$) and receiver operating characteristics curve analyses. On stepwise multinominal logistic regression analysis, serum A-FABP was a significant independent baseline predictor of CACS at 10 years (OR=2.04, 95% CI=1.22-3.43 for CACS 1-100 vs CACS=0, P=0.007; and OR=2.40, 95% CI=1.19-4.83 for CACS >100 vs CACS=0, P=0.014), together with age and male sex.

Conclusion: A-FABP may serve as a useful marker, in combination with current risk assessment tools, to identify individuals at increased risk of CHD.

Acknowledgement: This study was supported by the Hong Kong Research Grant Council (HKU7590/06M); Central Allocation (HKU 2/07C), and Hong Kong Innovation and Technology Fund (GHP/027/05).

Increased regulatory T cells are associated with liver injury in patients with chronic hepatitis B

59

YD Wang, HY Zhang, L Lu, J Li, DW Ye, A Wong, AYK Kwok, GKK Lau
Department of medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Chronic hepatitis B (CHB) infection is believed to be associated with ineffective antiviral T-cell response while the detailed mechanism remained poorly understood. Recent studies have suggested that regulatory T cells (Tregs) may contribute to immune regulation in patients with CHB infection, but their role in disease progress is still unclear. The aim of this study was to explore the correlation between the Tregs and the liver injury or viral replication level.

Methods: A total of 50 treatment-naïve CHB patients, including 30 in immune-tolerant phase (age: 23 [18-47] years; HBsAg and HBeAg positive; ALT: 20 [5-29] U/L; serum HBV DNA: $1.77x10^9$ [$6.30x10^6$-$1.04x10^{10}$] copies/mL) and 27 in immune-clearance phase (age: 33.5 [21-43] years; HBsAg and HBeAg positive; ALT: 220 [100-1530] U/L; serum HBV DNA: $8.52x10^8$ [$2.11x10^6$-$1.77x10^{10}$] copies/mL) were enrolled in the study. The frequency of Tregs ($CD4^+CD25^+Foxp3^+$ T cells) in PBMCs was analysed using flow cytometry. The level of Foxp3 gene expression in PBMCs was examined through real-time PCR.

Results: The frequency of Tregs was significantly higher in PBMCs from CHB patients in immune-clearance phase when compared with that in immune-tolerant phase (mean±SEM: 2.01±0.20% vs 1.53±0.13%, $P<0.05$, Student's *t* test) and a significant correlation was observed between the frequency of Tregs and serum ALT level in PBMCs from CHB patients in immune-clearance phase ($r=0.419$, $P<0.05$, Spearman correlation analysis). However, the frequency of Tregs did not correlate with serum HBV DNA level. The level of Foxp3 gene expression in PBMCs from CHB patients in immune-clearance phase was also significantly higher than that in immune-tolerant phase (1.84±0.34% vs 1.00±0.18%, $P<0.05$).

Conclusion: The frequency of Tregs was significantly different in CHB patients between immune-tolerant phase and immune-clearance phase. Increased Treg cells were associated with liver injury.

Acknowledgement: This work was supported by the grants from the 973 National Basic Research Program of China (2007CB512800), RGC (HKU 7679/06M) to Prof. George K.K. Lau and Cheng Si Yuan China-International Hepatitis Research Foundation.

Berberine as a new drug for the treatment of vascular complications in diabetes: role of AMP-activated protein kinase

60

YQ Wang[1], Y Huang[3], KSL Lam[1], CW Lau[3], PM Vanhoutte[2], AM Xu[1,2]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Pharmacology, The University of Hong Kong, Hong Kong
[3]Department of Physiology, The Chinese University of Hong Kong, Hong Kong

Introduction: Vascular disorder is a common soil for many deadly diseases and is one of the most common complications observed in type 2 diabetes. Endothelial dysfunction, characterised by impaired vasodilatation, is a key event that links obesity, diabetes, hypertension and vascular diseases. The AMP-activated protein kinase (AMPK) plays a key role in endothelial cell, including protection of cells from apoptosis, inhibition of inflammation, and stimulation of angiogenesis. The objectives of this study were to evaluate the protective effects of berberine, an alkaloid purified from traditional Chinese medicine, against hyperglycaemia-induced cellular injury, endothelial dysfunction, and investigate the potential role of AMPK pathway in this process.

Methods: Berberine was tested for its effects on the production of nitric oxide (NO), activation of eNOS as well as the association of eNOS with heat shock protein (HSP)90 and AMPK in human umbilical vein endothelial cells (HUVEC). The effect of berberine on vascular reactivity was examined on aortic rings isolated from rats. In addition, berberine was also evaluated for its actions on inhibiting production of intracellular ROS, apoptosis and NF-kappa B activation under hyperglycaemia circumstance.

Results: In cultured endothelial cells and blood vessels, berberine dose-dependently enhanced eNOS phosphorylation and promoted the association of eNOS with (HSP)90, leading to an increased production of NO. Furthermore, berberine attenuated high glucose-induced generation of reactive oxygen species (ROS), cellular apoptosis, NF-kappa B activation and expression of adhesion molecules, thus suppressing monocyte attachment to endothelial cells. In rat aortic rings, berberine elicited endothelium-dependent vasodilatation and alleviated ROS-mediated endothelial dysfunction. These beneficial effects of berberine on the endothelium were abolished by either pharmacological inhibition of AMPK, or adenovirus-mediated overexpression of a dominant negative version of AMPK.

Conclusions: Results of the present study demonstrate that berberine protects against endothelial injury and enhances NO-dependent vasodilatation through activation of the AMPK/eNOS signalling cascade. Berberine or its derivatives may be useful for the treatment and/or prevention of endothelial dysfunction associated with diabetes.

Acknowledgement: NSFC/RGC (N HKU 727/05) and the HKU matching funding for National "973" basic research project.

Effectiveness of western medicine out-patient consultations in primary care — a comparison with Chinese medicine consultations

61

W Wong, LK Lam, SW Ho, YZ Choi, HH Sze
Family Medicine Unit, Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Primary care plays an important role in the health care system, but research evidence on the effectiveness of primary care is largely lacking. This study aimed to determine the effectiveness of western medicine (WM) consultations and to compare it with that of traditional Chinese medicine (TCM) consultations in primary care in Hong Kong. This was a prospective, longitudinal study on 841 patients who consulted two WM public general out-patient clinics (GOPC) in Hong Kong for an episodic illness.

Methods: All patients attending the Tung Wah Hospital or Ap Lei Chau WM GOPC who fulfilled the inclusion criteria were invited to participate. Each patient answered a structured questionnaire on the Chinese Quality of Life instrument (ChQOL), the SF-36 Health Survey before and 2 weeks after the doctor consultation. The Global Rating on change Scale (GRS) was also administered in the week-2 assessment. The primary outcomes were changes in the ChQOL and SF-36 HRQOL scores. Secondary outcomes included the GRS score.

Results: The most common (26.2%) presenting problems to WM clinics were respiratory symptoms (c.f. 7.9% in TCM clinic study) and 9.5% were musculo-skeletal problems. 60% (c.f. 36% in TCM clinic study) of the subjects had not used other health services before consulting the WM GOPC. The mean ChQOL and SF-36 domain scores of subjects improved significantly 2 weeks after the consultations except for the physical form domain of ChQOL. The greatest improvements were found in the SF-36 physical health–related domains. 71% of subjects reported an improvement in global rating of change in their condition after WM consultations. The proportion of subjects who had improvement in HRQOL scores were lower among subjects consulting the WM clinic than those consulting clinics but the difference was not significant after correction for baseline scores.

Conclusions: More people used WM than TCM as the first primary care service. The morbidity patterns presenting to WM and TCM clinics were different, suggesting that they serve different patient needs. WM consultations were associated with significant improvement in HRQOL in over 90% of patients. There was no significant difference between the effectiveness of WM and TCM consultations. There is potential for TCM to play a more important role as an alternative primary care service in Hong Kong.

Molecular mechanism for the modulation of human ether á-go-go gene potassium channels by EGFR tyrosine kinase

62

W Wu, CP Lau, HF Tse, GR Li
Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Human ether á-go-go gene potassium channels (hEAG1) are expressed in brain and several types of human cancers, and play a critical role in neuronal excitement and tumour progression. However, functional regulation of hEAG channels is not understood. The present study was designed to determine whether hEAG1 channels are regulated by epidermal growth factor receptor (EGFR) kinase.

Methods: The hEAG1 current was recorded in HEK 293 stably expressing hEAG1 gene with a whole-cell patch clamp technique. Mutants of hEAG1 channels were generated using site-directed mutagenesis.

Results: It was found that AG556, a highly selective inhibitor of EGFR kinase, suppressed hEAG1 current in a concentration-dependent manner. The inhibitory effect was fully antagonised by co-application of AG556 and orthovanadate (1 mM, inhibitor of protein tyrosine phosphatases). In addition, EGF (100 ng/mL) slightly increased hEAG1 current in cells with a 36-hour starvation. In mutants of hEAG1 channels, the inhibitory effect of hEAG1 current by AG556 was largely attenuated for hEAG1-Y90A, Y344A and Y485A, but not for hEAG1-Y376A, Y479A and Y639F.

Conclusion: Our results demonstrate for the first that EGFR kinase modulates hEAG1 channel activity via phosphorylating tyrosine residues (Tyr^{90}, Try^{344} and Try^{485}) and therefore likely regulates neuronal activity and tumour growth.

Acknowledgement: This research was supported by Sun Chieh Yeh Heart Foundation.

63

Differential roles of Smads in angiotensin II–induced tubular epithelial-mesenchymal transition

FY Yang[1], XR Huang[1], ACK Chung[1], CC Hou[2], KN Lai[1], HY Lan[1]
[1]Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Medicine-Nephrology, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan

Introduction: Angiotensin II (Ang II) has been demonstrated to mediate epithelial-mesenchymal transition (EMT) by activating the TGF-β/Smad signalling pathway. The present study dissected the specific role of Smads in Ang II–induced EMT in vivo and in vitro.

Methods: Ang II–induced EMT in a normal rat tubular epithelial cell line (NRK52E) was determined by a loss of E-cadherin, an epithelial marker, and de-novo expression of alpha-smooth muscle actin (alpha-SMA), a mesenchymal marker by real-time PCR, western blot and immunohistochemistry analyses. Ang II–induced activation of Smad signalling via the Smurf2mediated Smad7 ubiquitin-proteasomal degradation pathway was investigated by knocking down Smurf2 with siRNA technique. Specific role for Smad2, Smad3, or Smad7 in Ang II–induced EMT was dissected by knocking down Smad2, Smad3 and overexpressing Smad7.

Results: We found that Ang II signalled through the receptor of AT1, not AT2, to activate Smad signalling and induce EMT in NRK52E. Ang II–induced EMT was negatively regulated by Smad7 because overexpression of Smad7 was able to block Ang II–induced Smad2/3 activation and EMT in vitro in an inducible Smad7 expressing tubular epithelial cell line and in vivo in a rat model of remnant kidney disease by gene transfer of Smad7. Activation of Smad signalling by Ang II was associated with degradation of Smad7, which was mediated by the AT1-Smurf2-ubiquitin-degradation mechanism because addition of antagonist to AT1 receptor (losartan) and siRNA to Smurf2 was able to block Ang II–induced upregulation of Smurf2, thereby preventing Smad7 from degradation. Further studies showed that Ang II–induced EMT was mediated by Smad3, but not Smad2 because knockdown Smad3, not Smad2, blocked EMT in response to Ang II.

Conclusions: Ang II was able to induce EMT by activating the Smad signalling pathway via the Smurf2-Smad7 ubiquitin degradation pathway. Ang II induces EMT positively by Smad3, not Smad2, but negatively by Smad7.

Acknowledgement: This study was supported by grants from Research Grant Council of Hong Kong (RGC GRF 759206 and 768207 to HYL).

64

Association of hepatitis B virus Pre-S mutations with the risk of hepatocellular carcinoma development

AP Yeung, DKH Wong, J Fung, JCH Yuen, FKC Fung, CL Lai, MF Yuen
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: Recent studies suggest that hepatitis B virus (HBV) Pre-S/S mutations are associated with the development of hepatocellular carcinoma (HCC). However, in these case-control studies, the patients were not matched for age, gender and hepatitis B e-antigen (HBeAg) status. We aimed to investigate the association between PreS deletions and HCC using (1) a matched case-control approach and (2) a longitudinal approach.

Methods: HBV PreS deletions were determined by DNA sequencing in sera collected from 105 HCC and 105 non-HCC patients matched with age, gender and HBeAg status, as well as in sera collected before the development of HCC.

Results: PreS deletions were detected in 27 of 105 HCC cases (25.7%). At the time of writing, nucleotide sequence analysis in 68 HCC/non-HCC–matched pairs showed that 20 HCC (29.4%) and 9 non-HCC (13.2%) patients acquired PreS2 deletions (P=0.035). In the longitudinal study, serum samples collected 1 to 7 years before HCC development were assessed in 12 HCC cases with PreS deletions. PreS deletions were absent in seven cases before HCC development (58.3%).

Conclusion: The findings from this preliminary study suggested that PreS deletions, especially PreS2 deletions, were associated with HCC development. These results are being validated by our on-going studies with a larger number of patients.

rs7826463 Polymorphism of the adipocyte-fatty acid-binding protein (A-FABP) gene is independently associated with hypertension

65

DCY Yeung[1,3], BMY Cheung[1], A Xu[1,3], WS Chow[1], CHY Fong[1], KL Ong[1], TC Pun[2], PC Sham[4], KSL Lam[1,3]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Obstetrics and Gynaecology, The University of Hong Kong, Hong Kong
[3]Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Hong Kong
[4]Genome Research Centre, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Adipocyte-fatty acid-binding protein (A-FABP) is an adipokine regulating systemic insulin sensitivity, lipid and glucose metabolism. It is secreted from the adipocytes and circulates in the human bloodstream. Previous cross-sectional and prospective studies have revealed that serum A-FABP levels are closely associated with adiposity, metabolic syndrome, type 2 diabetes, carotid atherosclerosis, and diabetic nephropathy. This study aimed at identifying A-FABP polymorphisms that can be used as genetic markers for obesity-related medical complications.

Methods: Four tagging SNPs (rs7826463, rs2290200, rs10808846, and rs1486006) were selected from the HapMap Han Chinese (Beijing) of the International Haplotype Map Project database and were genotyped. Two proximal promoter polymorphisms (rs16909225 and rs3834363) and two SNPs (rs3841580 and rs16909196) reported in the NCBI dbSNP database but not the HapMap were also genotyped. Genotype-phenotype associations were calculated in 1938 subjects, who returned for follow-up for the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS) and had available DNA samples. Protein levels of A-FABP were measured by enzyme-linked immunosorbent assay (ELISA) in another 73 women who underwent benign gynaecological operations at the Queen Mary Hospital, and correlated with the rs7826463 genotype of these subjects.

Results: Individuals carrying the variant T allele of rs7826463 had significantly reduced systolic and diastolic blood pressures (both $P<0.03$) as well as a lower prevalence of hypertension ($P=0.01$). On multivariate analysis, T allele carriers had a significantly reduced odds ratio of hypertension (OR=0.63; 95% CI, 0.42-0.95; $P=0.026$), while age, waist circumference and HOMA insulin resistance index conferred increased risks. Visceral fats from the T allele carriers showed a trend towards a lower level of A-FABP protein expression ($\beta= -0.342$, $P=0.089$).

Conclusion: T allele of rs7826463 of the A-FABP gene had a protective effect against hypertension.

Acknowledgement: This study was supported by the Hong Kong Research Grant Council (7590/06M).

Gastrointestinal decontamination for deliberate drug overdose: experience in an Emergency Department of a tertiary regional hospital in Hong Kong

66

WL Yip[1], SH Tsui[1], CR Kumana[2]
[1]Accident and Emergency Department, Queen Mary Hospital, Hong Kong
[2]Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Gastric lavage is no longer advocated as part of the routine management of patients taking a deliberate drug overdose by the oral route. We therefore set out to examine recent trends in gastrointestinal decontamination and outcomes in such patients presenting to our accident and emergency (A&E) department.

Methods: Data about patients presenting to A&E following deliberate drug overdose by the oral route during the inclusive periods 1998-1999 and 2006-2007, were reviewed and compared.

Results: Numbers of such patients presenting to A&E during these periods, the corresponding types of gastrointestinal decontamination they received, and in-hospital deaths are shown in the Table.

	Total	Activated charcoal	Gastric lavage	Syrup of ipecacuanha	Whole bowel irrigation	No gastrointestinal decontamination	Death
1998	299	63	73	0	0	203	2
1999	416	159	79	5	0	245	5
Total	715	222 (31%)	152 (21%)	5 (1%)	0 (0%)	448 (63%)	7 (1%)
2006	324	95	21	0	1	226	4
2007	343	91	7	1	1	245	4
Total	667	186 (28%)	28 (4%)	1 (0.1%)	2 (0.3%)	471 (71%)	8 (1%)

Conclusions: Whilst activated charcoal only was the commonest type of gastrointestinal decontamination deemed necessary, most of these patients did not receive any form of decontamination. There was a marked trend away from gastric lavage in recent years, without any change in in-hospital mortality.

A prospective multi-centred clinical study of photorejuvenation in Asian skin using 2790 nm infrared laser

67

CS Yu, K Negishi, SYN Shek, NPY Chan, HHL Chan
Department of Medicine, The University of Hong Kong, Queen Mary Hospital, Hong Kong

Introduction: This was a prospective multi-centred clinical study to evaluate the safety and efficacy of the 2790 nm Er:YSGG laser for resurfacing in Asians.

Methods: A total of 41 Asian subjects with Fitzpatrick skin types III-V and moderate-to-severe photodamage were recruited. Two full facial treatments were performed at 4-week intervals. A 2790 nm μm laser was used with settings of 2.0 J/cm^2 fluence, 0.4 ms pulse duration, single pass and 20% overlap. Standardised photographs were taken at baseline, 1 and 3 months after the second treatment. Two blinded assessors evaluated the photographs to assess the degree of improvement in fine lines, skin texture, irregular pigmentation, pigment spots, pore size and telangiectasia on a scale of 0 (no improvement) to 4 (excellent improvement). Patient satisfaction scores were also obtained. Cutometry was performed at five standard anatomical points on each visit.

Results: All subjects tolerated the procedure well using topical anaesthesia. Erythema and desquamation occurs after a mean of 3.4 and 6.7 days, respectively. Objective evaluation documented statistically significant improvement in terms of irregular pigmentation, pore size, and telangiectasia ($P<0.05$). 90.2% of subjects reported moderate-to-excellent overall improvement. Cutometry showed statistically significant improvement in skin elasticity at all points. There were two cases of mild post-inflammatory hyperpigmentation (4.9%), and one case of herpes simplex infection.

Conclusion: The 2790 nm Er:YSGG laser appears to be safe and effective for photorejuvenation in Asians with minimal adverse sequelae. However, further studies are necessary to optimise treatment parameters.

Inactivation of hypoxia inducible factor (HIF) 1 alpha induces obesity-associated metabolic disorders through brown adipose tissue dysfunction

68

XM Zhang[1,2], KSL Lam[1,2], MY Zhou[1,2], JL Chang[1,2], SK Chung[2,3], AM Xu[1,2]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Research Centre of Heart, Brain, Hormone and Healthy Aging, The University of Hong Kong, Hong Kong
[3]Department of Anatomy, The University of Hong Kong, Hong Kong

Introduction: Obesity is the major risk factor for a cluster of metabolic and cardiovascular diseases. Although the molecular events that link obesity with its related disorders remain poorly understood, growing evidence suggests that inflammation might be a key 'culprit'. Adipose tissue is now recognised to be one of the major contributors of systemic inflammation observed in obese states. Recent studies have demonstrated that relative hypoxia may contribute to obesity-induced inflammation, insulin resistance and other metabolic and cardiovascular disorders by activating hypoxia inducible factor 1 alpha (HIF 1 alpha), a transcription factor critically involved in the regulation of inflammation. However, the role of HIF 1 alpha in adipose tissues is still unclear. This study investigated the role of HIF 1 alpha in obesity-induced metabolic disorders in adipose tissues.

Methods: Transgenic mice with adipose tissue–specific over-expression of dominant negative (DN) HIF 1 alpha were generated. Their phenotypic changes under high fat diet were comprehensively characterised. Adipose tissues, liver, muscle and serum were collected for further biochemical and morphological analysis.

Results: Adipose tissue–selective inactivation of HIF 1 alpha mice developed more severe obesity compared to their littermate control, and exhibited hyperglycaemia and hyperinsulinaemia, causing the impairment of glucose tolerance and insulin sensitivity. Histological analysis showed that brown adipose tissue disappeared in the transgenic mice, leading to decreased energy dissipation. Real-time PCR demonstrated that the expression of gene encoding mitochondria proteins was decreased in the transgenic mice, which was accompanied by decreased copy number and protein content of mitochondria.

Conclusions: Adipose tissue selective inactivation of HIF 1 alpha accelerates obesity development by increasing fat mass, possibly through the mitochondrial dysfunction in brown adipose tissue.

Acknowledgement: This research was supported by RGC 764506M.

Cardiac Nav1.5 channels are modulated by epidermal growth factor receptor tyrosine kinase

69

YH Zhang, CP Lau, HF Tse, GR Li
Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Nav1.5 is the pore-forming α-subunit protein of the cardiac sodium channels which plays a pivotal role in the initiation and propagation of the cardiac action potential. It is generally believed that cardiac sodium current (I_{Na}) is regulated by protein phosphorylation.

Methods: The present study was designed to determine whether protein tyrosine kinases (PTKs) regulate human cardiac Nav1.5 channels stably expressed in HEK 293 cells using a whole-cell patch clamp technique.

Results: It was found that human cardiac I_{Na} was enhanced by epidermal growth factor (EGF, 100 ng/mL) or the protein tyrosine phosphatases (PTPs) inhibitor orthovanadate (1 mM). The selective EGFR kinase inhibitor AG556 (5 μM) remarkably inhibited I_{Na} amplitude, shifted the inactivation voltage toward negative potentials, and slowed the recovery of I_{Na} from inactivation. These effects were antagonised by orthovanadate. However, insulin and the Src-family tyrosine kinase inhibitor PP2 had no significant effect on I_{Na}.

Conclusion: These results suggest that EGFR kinase and PTPs regulate human cardiac Nav1.5 channels stably expressed in HEK-293 cells. EGFR kinase positively, while PTPs negatively modulates the channels. Additional experiments are required to confirm tyrosine phosphorylation level of Nav1.5 using immunoprecipitation and western blot analysis and to find out the tyrosine phosphorylation site(s) of Nav1.5 by site-directed mutagenesis.

Mice deficient for P53 are protected against acute aristolochic acid nephropathy

70

L Zhou, P Fu, XR Huang, F Liu, R Zhang, KN Lai, HY Lan
Department of Medicine, The University of Hong Kong, Hong Kong

Introduction: Aristolochic acid (AA) is a traditional Chinese medicine with anti-inflammatory properties and has been shown to be a key mediator of aristolochic acid nephropathy (AAN) characterised by progressive tubular epithelial cell (TEC) loss and renal scarring. However, the mechanisms by which AA induces AAN remain largely unknown. We have previously shown that AA acts by stimulating p53 to induce TEC death through apoptosis. The present study tested the functional role of p53 in the pathogenesis of acute AAN in p53 knockout mice.

Methods: Acute AAN was induced in p53 wild type (WT) and knockout (KO) mice by a daily intraperitoneal injection of AA at a dose of 10 mg/kg for 3 days. Histologic study, serum creatinine, creatinine clearance rate, protein in urine, TUNEL staining and western blot analysis were used to evaluate the renal damage and apoptosis.

Results: Compared to normal mice, 3 days after AA injection, WT mice developed acute AAN as demonstrated by a 2.5-fold increase in serum creatinine ($P<0.01$), a 2.5-fold fall in creatinine clearance ($P<0.01$), and severe acute tubular necrosis/apoptosis (23% increase). These acute kidney injuries were mediated by p53 because AA caused a marked phosphorylation of p53 and cleaved caspase-3, resulting in a 3-fold increase tubular cell apoptosis (TUNEL+cells). In contrast, mice lacking p53 were protected against the development of acute AAN. This was demonstrated by preserving normal levels of serum creatinine and creatinine clearance and largely reduced the severity of tubular apoptosis as evident by inhibiting cleaved caspase-3 (60% reduced) and $TUNEL^+$ cells (a 2-fold reduce compared to AAN WT mice).

Conclusion: AA may act by the p53 pathway to induce acute AAN via apoptosis. Thus, activation of the p53 pathway may be a central mechanism of acute AAN.

Adiponectin exerts its hepatoprotective functions against fatty liver disease through regulating mitochondria activities

71

M Zhou, A Xu, PK Tam, KS Lam, L Chan, RL Hoo, J Liu, KH Chow, Y Wang
Department of Medicine and Department of Pharmacology, The University of Hong Kong, Hong Kong

Introduction: Adiponectin is an adipocyte-derived hormone possessing a wide range of beneficial effects on obesity-related medical complications. Numerous epidemiological investigations in diverse ethnic groups have identified lower adiponectin level as an independent risk factor for non-alcoholic fatty liver diseases and liver dysfunctions. Animal studies have demonstrated that replenishment of adiponectin protects against various forms of hepatic injuries, suggesting it to be a potential drug candidate for the treatment of liver diseases. This study was designed to investigate the cellular and molecular mechanisms underlying the hepatoprotective effects of adiponectin.

Methods: The hepatic mitochondrial respiration chain (MRC) activities of C57BL/6J and adiponectin knock out (ADN-KO) mice of the same background were measured by biochemical assays. Replenishment of adipoenctin was achieved through recombinant adenovirus-mediated overexpressions. Liver steatosis was evaluated by Red Oil O staining. Lipid peroxidation products were monitored by measuring the malondialdehyde levels. Quantitative PCR was applied to examine different gene expressions.

Results: Our results demonstrated that in ADN-KO mice, there was a pre-existing condition of hepatic steatosis and mitochondria dysfunction, which might contribute to the increased vulnerabilities of these mice to secondary liver injuries induced by obesity and other conditions. Adenovirus-mediated replenishment of adiponectin depleted lipid accumulation, restored the oxidative activities of MRC complexes, and prevented the accumulation of lipid peroxidation products in ADN-KO mice, but had no obvious effects on mitochondria biogenesis. The gene and protein levels of uncoupling protein 2 (UCP2), a mitochondria membrane transporter, were decreased in ADN-KO mice and could be significantly up-regulated by adiponectin treatment. Moreover, the effects of adiponectin on mitochondria activities were partially abolished in UCP2 knockout mice.

Conclusion: These results suggest that the hepatoprotective properties of adiponectin are mediated at least in part by an enhancement of the activities of MRC complexes through a mechanism involving UCP2.

Acknowledgement: This research was supported by Seeding Funds for Basic Research of the University of Hong Kong; Hong Kong Research Grant Council; Grant Number: HKU 778007, HKU 7645/06M; U.S. National Institutes of Health; Grant Number: HL-51586; Area of Excellent Scheme established under the University Grants Committee of the Hong Kong Special Administrative Region; Grant Number: AoE/P-10-01.

Multiple ion channels inhibition contributes to arrhythmogenic effect of chloroform

72

Y Zhou[1,2], TM Wong[1], GR Li[1,2]
[1]Department of Physiology, Faculty of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Medicine[2], Faculty of Medicine, The University of Hong Kong, Hong Kong

Introduction: Chloroform, a widely used organic solvent in industrial production, is found to cause intoxication of lethal arrhythmias. Since the ionic mechanisms of its arrhythmogenic effects are still unknown, the present study was designed to investigate the electrophysiological basis of the arrhythmogenic effect of chloroform.

Methods: A whole-cell patch clamp technique was employed to study effects of chloroform on HEK 239 cells stably expressing human cardiac inward rectifier K+ channel (Kir2.1), human cardiac ether-a-go-go related (hERG) K+ gene, Nav1.5, and pacemaker gene (HCN2). The effect of chloroform on isolated rat heart was also studied.

Results: We found that chloroform showed an obvious arrhythmogenic effect in isolated rat heats at the concentration of 10 mM. It also inhibited the pacemaker HCN2 channel and human cardiac IKr (ie hERG) channels in a concentration-dependent manner, with IC50 of 3.39 mM and 4.29 mM respectively. The inhibition of chloroform leftward shifted hHCN2 activation curve and the activation kinetics. In addition, chloroform inhibited Nav1.5 currents by 24.5%, 47.6%, and 82.8% at 5, 10 and 15 mM, respectively. However, chloroform (10 mM) had no effect on human cardiac Kir2.1 channels.

Conclusion: Inhibition of multiple cardiac ion channels, including IKr, INa, and the pacemaker HCN2, likely contributes to at least in part for the chloroform-induced lethal arrhythmias, and thus these findings may be helpful in seeking effective management of acute chloroform intoxication.

73

APPL1 and APPL2 antagonise each other in regulating cytokinesis through MgcRacGAP

W Zhu[1], KK Cheng[1], Y Wang[2], KSL Lam[1], A Xu[1,2]
[1]Department of Medicine, The University of Hong Kong, Hong Kong
[2]Department of Pharmacology, The University of Hong Kong, Hong Kong

Introduction: APPL1 and APPL2 are two intracellular adapter proteins containing a PH domain, a PTB domain, and a Leucine zipper motif. Recent studies have found that APPL1 acts as a key signalling molecule integrating multiple signalling stimuli. However, the cellular functions of APPL2 and its relationships with APPL1 remain poorly understood. The objective of this study was to apply proteomics-based approaches to identify intracellular binding partners of these two adaptor proteins, which may in turn provides important clues for cellular functions of APPL1 and APPL2.

Methods: Proteins physically associated with APPL1 or APPL2 were retained by pull-down purification and co-immunoprecipitation, followed by mass spectrometry-based proteomic identification. Binging sites of APPL2 with MgcRacGAP were determined by site-directed mutagenesis. Effects of APPL1 and APPL2 in regulating cell proliferation were measured by flow cytometry analysis, and cellular distribution of proteins were visualised by immunocytochemistry and observed by confocal microscopy.

Results: Western blot analysis revealed that these two proteins have distinct expression patterns. Proteomic analysis showed that these two proteins interact with each other; on the other hand, each of these two proteins binds to a distinct set of signalling components. Truncated segment binding assay also identified the interaction domains of APPL2 and MgcRacGAP. Furthermore, we demonstrated that these two proteins have antagonistic effects in regulating cytokinesis through regulating the intracellular localisation of MgcRacGAP. APPL2 inhibits cytokinesis through interacting with MgcRacGAP, while APPL1 promotes cytokinesis by blocking the association of APPL2 with MgcRacGAP.

Conclusion: Our data suggest that APPL1 and APPL2 might act as a pair of 'Yin-and-Yang' molecules critically involved in the regulation of cytokinesis and other multiple signalling pathways. Further investigations on these two proteins might lead to the identification of novel regulatory mechanisms involved in cell growth and development.

Acknowledgement: This research was supported by RGC (779707M to Xu A) and matching fund from National 973 basic Research project.

AUTHOR INDEX