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Plenary 1

Bypassing pluripotency

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Heart disease is the leading cause of morbidity and mortality in developed countries. Cardiomyocytes are terminally differentiated cells and their regenerative capacity is quite limited in the adult heart. During the past several years, discovery of iPS cells and significant advances in iPS cell generation technology, cardiac differentiation, and cell purification protocols were achieved for the development of stem cell–based heart therapies. The generation of iPS cells by combination of transcription factors has also sparked a new approach which is direct conversion of mature cell types into another cell type without passing through a pluripotent stem cell state. Functional cardiomyocytes can be directly reprogrammed from differentiated somatic cells by transduction of the three cardiac transcription factors, Gata4, Mef2c and Tbx5, in vitro.[1] Moreover, gene transfer of the cardiac reprogramming factors could convert cardiac fibroblasts into cardiomyocyte-like cells in mouse infarct hearts. A new polycistronic retrovirus expressing GMT separated by 2A self-cleaving peptides induced multiple cardiac gene expression, cardiac proteins, and new cardiomyocyte-like cells in infarct hearts.[2] More recently, we found that addition of Myocd and Mesp1 to GMT (GMTMM) directly reprogrammed human fibroblasts into cardiomyocyte-like cells in vitro.[3] I will overview the recent research achievements and discuss future challenges of direct cardiac reprogramming for heart regeneration.

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Plenary 2

Cardiac regeneration: stem cells and beyond

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Myocardial infarction, blockage of a coronary artery, leads to deprivation of oxygen in a part of the heart muscle, and irreversible loss of cardiomyocytes. Since cardiomyocytes are unable to proliferate sufficiently, the damaged contractile tissue is replaced by a rigid scar, thereby diminishing the pump function of the heart. This will further attenuate cardiac contractility and ultimately result in heart failure. Novel approaches to ameliorate or even reverse the progression of heart failure, include the use of progenitor or stem cells with the ability to differentiate into new cardiac tissue. We and others have shown that multipotent cardiac stem / progenitor cells reside in the heart that can differentiate into cardiac myocytes, smooth muscle cells, and vascular endothelial cells after transplantation into the injured myocardium, but due to the low retention of cells, this strategy has thus far had limited impact on cardiac function. Endogenous regeneration of the mammalian neonatal heart, and the discovery that it may still persist in adulthood sparked hope for novel cardioregenerative therapies. In this talk, I will give an overview of the current options to restore the contractile force of the heart: the different stem cell sources as therapeutic agents in cardiac repair as well as more novel approaches like the activation of endogenous cell populations, the use of paracrine factors and exosomes, as well as their use in preclinical and clinical studies to repair the injured myocardium.

Can we use the immune system to conquer cancer?

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The journal *Science* has voted cancer immunotherapy as the most important scientific breakthrough of 2013. Recent successful phase III clinical trials are testament to the relevance of the immune system in fighting human cancer.

In a phase II clinical study, we treated 21 patients with heavily pretreated metastatic nasopharyngeal cancer (NPC) with non-myeloablative blood stem cell transplant (NST) using HLA-matched and 1-antigen mismatched sibling peripheral blood stem cell allografts. We demonstrate for the first time that NST can induce meaningful clinical responses in patients with advanced NPC with prolonged disease control achieved in some patients.

We also present a first-in-man clinical trial of an intradermal autologous dendritic cell (DC) cancer vaccine transduced with replication-deficient adenoviral vector Ad5f35 encoding truncated LMP1 and full-length LMP2 in 16 patients with pretreated metastatic NPC who have failed one or more lines of treatment and another lysate-pulsed DC vaccine trial in refractory colorectal cancer.

Autologous EBV-antigen specific cytotoxic T lymphocytes (CTL) can be activated, expanded and adoptively transferred as a cell-based immunotherapeutic strategy against virally transformed cancers. We developed an adoptive cell therapy strategy of serial infusions of autologous EBV-specific CTL for first-line treatment of advanced NPC patients following a course of potentially synergistic combination chemotherapy with gemcitabine + carboplatin. This phase II study has completed accrual of all 38 patients with advanced NPC and interim clinical, biomarker correlates and translational results will be presented for discussion. With a median follow-up of 2 years, median overall survival was 29.9 months and I-year and 2-year survival were 77% and 63%, respectively. These outcomes represent the most positive results of any systemic therapy against advanced NPC. I will provide an update of this mature, completed study and its translational aspects.

I will present an overview and update of the latest global cutting edge developments of immune-based studies that have been proven to prolong survival in phase III clinical trials, including those that influence immune checkpoint processes such as anti-CTlA4Ig and anti-PD1 therapies, and provide an overall landscape of the latest in immunotherapy against cancer.

The efficacy of a microwave device for treating hyperhidrosis in Chinese

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Objective: Hyperhidrosis affects the quality of lives. A non-invasive, microwave device selectively heats the targeted region where the sweat gland resides. The objective of the study was to assess the efficacy and patient satisfaction after treatment.

Methods: Ten Chinese subjects with skin type III-IV with axillary hyperhidrosis were enrolled. Subjects were required to have a Hyperhidrosis Disease Severity Scale (HDSS) score of 3 or 4 at baseline. Two treatments were offered at 3 months apart. Efficacy was assessed using HDSS and Dermatology Life Quality Index (DLQI) at baseline and every month until the sixth visit at 3 months post–second treatment. Responders are those achieving HDSS score of 1 to 2.

Results: All subjects received the first treatment and three subjects received the second treatment. Nine subjects had 1-month follow-up, all of which reported an HDSS score of 1 (33%) and 2 (67%). In terms of the DLQI, the score has significantly improved at 1 month ($P = 0.008$) at 1-month post-treatment. All subjects experienced transient swelling and one subject reported mild numbness.

Conclusion: The initial results demonstrate that the microwave device is promising for treating axillary hyperhydrosis.

Non-invasive cryolipolysis for fat reduction in flanks in Chinese with a modified applicator

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Objective: To determine the patient satisfaction and clinical efficacy of a modified applicator with a cryolipolysis device (Zeltiq®) for fat reduction in the flanks in Chinese.

Methods: A total of 15 healthy adult subjects with clearly visible fat in the flanks that wish to have them reduced were recruited. All received a single free treatment. Parameters were pre-set at cooling intensity factor of 41.6 (-73 mW/cm^2) for 60 minutes per site. The efficacy is determined by comparing measurements and comparing photographs taken at baseline, and 8 weeks and 12 weeks post-treatment. Blinded independent reviewers assessed the standardised photographs taken by the Canfield System®. The fat thickness was recorded by a handheld caliper and subject satisfaction assessed by questionnaires were collected. In addition, a questionnaire based on the fit of the new applicator had to be filled in by the operator at the treatment visit. Any incidence of device or procedure-related adverse effects was recorded.

Results: Of the 15 subjects, 10 were satisfied at the 12-week post-treatment visit. In terms of fat thickness by caliper measurement, there was statistically significant difference ($P < 0.05$) between baseline and follow-up visits. The weights of the subjects were stable and there were no significant change in fat thickness at the control sites. Operator feedback showed that the new applicator has a better fit for Chinese patients and attaches easier than the original applicator. No adverse effects were recorded at 12-week post-treatment visit.

Conclusion: The new applicator for the treatment of fat bulge at the flanks is efficacious in Chinese. The modification of the applicator has a better fit ergonomically and has high operator satisfaction.

Safety and efficacy evaluation of a combined device using infrared light and bipolar radiofrequency and sublative radiofrequency applicators

3

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Objective: The combined infrared light and bipolar frequency and sublative radiofrequency dual handpiece device is intended for dermatological procedures requiring ablation and resurfacing of the skin. The objective of this study was to evaluate the safety and efficacy of the combined sublative radiofrequency and sublime applicators for the treatment of wrinkles.

Methods: Twenty subjects were recruited of whom 16 received the treatment and were followed up till 3 months. Each patient was treated once, first with the sublime applicator which used a combination of infrared (700-2000 nm) and bipolar radiofrequency energy; followed by the sublative radiofrequency handpiece, 2 passes of 65 mJ with a 64 pin tip and 30 mJ with a 30 pin tip for different areas. Objective assessment was performed by evaluation of standardised photography by independent physicians. Subjective assessment was performed by the study investigator based on subjective Global Aesthetic Improvement scale and grade the severity of wrinkling based on the Fitzpatrick Classification and elastosis score. The subjects were asked to complete a questionnaire.

Results: All subjects experienced moderate-to-severe pain during treatment. Other adverse effects include swelling, erythema, heat sensation, and crusting. From the preliminary data, three out of 11 subjects felt that there was an increase in pigmentation at 3-month follow-up. They noticed subjective improvements in skin texture, pore size, pigmentation, and skin laxity. The improvement in wrinkles seemed to be the most obvious. Investigator assessment showed statistically significant improvement in Fitzpatrick Wrinkle Scale ($P = 0.025$ and 0.014) and degree of elastosis score ($P = 0.005$ and 0.005) at 6 weeks and 3 months post-treatment, respectively.

Conclusion: From the preliminary data, there is subjective improvement in photoaged skin by the combined device using infrared light and radiofrequency and sublative radiofrequency applicators.

Efficacy of a high-intensity focused ultrasound device for non-invasive body contouring

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Objective: High-intensity focused ultrasound (HIFU) is a non-invasive technology for body contouring. HIFU is focused within the subcutaneous adipose tissue, causing coagulative necrosis and cell death. The objective of this study was to evaluate the effectiveness of a HIFU device for sculpting of the abdomen.

Methods: The system has a set focal depth of 1.3 cm. Twelve subjects with adipose thickness of no less than 2.5 cm who met the screening criteria were recruited. Each subject received one treatment to the abdomen. The total fluence used per site was 150-165 J/cm^2 with a mean of 161 J/cm^2. The waist circumference at iliac crest and the point of maximum circumference were recorded at baseline, and 4, 8, and 12 weeks post-treatment, as well as their weight and body mass index. Subjects' rating on comfort level and satisfaction were collected via questionnaires at every follow-up. Standardised photographs were also taken with the Canfield System® at each visit.

Results: Of the 12 subjects, seven were satisfied with the outcome and nine would recommend this treatment to their friends and family. There was statistically significant improvement in the waist circumference measured at both the iliac crest ($P = 0.013$, 0.002, and 0.005) and maximum waistline ($P = 0.003$, 0.034, and 0.023) at 4, 8, and 12 weeks post-treatment, respectively. Spearman's rho for correlation of energy level versus improvement showed that at 12 weeks' post-treatment follow-up, the improvement significantly correlated with the total fluence per treatment ($P = 0.041$). The higher the total fluence delivered, the larger the decrease in waist circumference.

Conclusion: HIFU effectively decreases waist circumference in Chinese. The higher the total fluence delivered, the larger the decrease in waist circumference was observed.

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Association between body mass index and cause-specific mortality as well as hospitalisation in Chinese older adults with multiple comorbidities

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Background: U-shaped relationship between body mass index (BMI) and all-cause mortality has been reported but there is little research examining the association between BMI and cause-specific mortality as well as hospitalisation. We performed a longitudinal study to examine the associations between BMI and cause-specific mortality and hospitalisation in Chinese older adults with multiple comorbidities, which could provide a reference for recommended BMI in this population.

Methods: It was a retrospective cohort for Chinese older adults of a regional Geriatric Day Hospital in Hong Kong from 2004 to 2013. They were divided into groups according to their BMI: <16; 16-18; 18.1-20; 20.1-22; 22.1-24; 24.1-26; 26.1-28; 28.1-30, and >30 kg/m^2. Other assessments included medical, functional, cognitive, social, and nutritional assessment.

Results: A total of 1747 older adults were included (mean age, 80.8 ± 7.1 years; 44.1% male; 46.1% living in nursing home; Charlson comorbidity index, 2.0 ± 1.6) with a median follow-up of 3.5 years. Older adults with BMI of 24-28 kg/m^2 had the lowest all-cause, infection-related, and cardiovascular mortality ($P < 0.001$). Multivariate analysis showed there was an inverted J-shape association between BMI and hazard ratio for all-cause and infection-related mortality, in both nursing home older adults and community-dwelling older adults. Rate of all-cause hospitalisation was lower in older adults with BMI of 22-28 kg/m^2 ($P = 0.002$). Multivariate analysis showed there was an inverted J-shape association between odds ratio of recurrent hospitalisation and BMI.

Conclusion: Chinese older adults with BMI of 24-28 kg/m^2 had lower all-cause mortality, infection-related mortality, cardiovascular-related mortality, and all-cause hospitalisation. This study provided a reference for recommended BMI in this population.

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Effectiveness of influenza vaccination in institutionalised older adults: a systematic review

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Background: Influenza infection is common among institutionalised older adults. Many non-randomised observational studies on influenza vaccination suggested that it could reduce influenza-related hospitalisations and mortality in institutionalised older adults. Criticism regarding the effectiveness of influenza vaccine estimated by non-randomised observational studies includes the frailty selection bias and use of non-specific outcome like all-cause mortality. A systematic review of studies of influenza vaccination in institutionalised older adults to determine the effects on clinical outcomes was conducted.

Methods: We searched for studies from three databases from 1946 to June 2013 assessing effectiveness against influenza infection. During selection process, we selected studies with well comparability between vaccine group and control group. We expressed vaccine effectiveness (VE) as a proportion, using the formula VE = 1–relative risk or 1–odds ratio. We focused on the following outcomes: influenza-like illness (ILI), laboratory-confirmed influenza, hospitalisations due to ILI or pneumonia and death due to influenza or pneumonia. All-cause mortality was not included.

Results: Eleven studies that satisfied the inclusion criteria were identified, representing 11 262 institutionalised older adults. After meta-analysis, we found a significant reduction in pneumonia (VE = 37%; 95% confidence interval [CI], 18-53%; $P = 0.001$) and death due to pneumonia or influenza (VE = 34%; 95% CI, 10-53%; $P = 0.01$). There was no significant heterogeneity between studies. There was no significant publication bias.

Conclusion: Influenza vaccination in institutionalised older adults could reduce pneumonia and death due to pneumonia or influenza. Influenza vaccination is recommended for institutionalised older adults.

Validation study of Charlson comorbidity index in predicting mortality in Chinese older adults

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Background: Charlson comorbidity index (CCI) is commonly studied for predicting mortality but there is no validation study of it in Chinese older adults.

Methods: We conducted a retrospective cohort study from 2004 to 2013 for patients discharged from a Geriatric Day Hospital in Hong Kong. Comorbidity was quantified using CCI and patients were divided into six groups according to their score of CCI: CCI-0, CCI-1, CCI-2, CCI-3, CCI-4, and CCI≥5. Other data collected included demographics, and functional, nutritional, cognitive, and social assessment. Outcome measure was 1-year mortality.

Results: At 1-year follow-up, 3.8% (n = 17), 5.9% (n = 37), 9.2% (n = 35), 12.9% (n = 20), 16.9% (n = 23), and 19.3% (n = 60) of CCI-0, CCI-1, CCI-2, CCI-3, CCI-4, and CCI≥5 died, respectively (P < 0.001). Multivariate analysis showed that CCI-1, CCI-2, CCI-3, CCI-4, and CCI≥5 had a hazard ratio (HR) of 1.34 (confidence interval [CI], 1.04-2.12), 2.18 (CI, 1.03-4.61), 3.44 (CI, 1.52-7.81), 3.74 (CI, 1.35-10.39), and 4.63 (CI, 2.28-9.43) respectively, compared with CCI-0. The area-under-curve of the receiver operating characteristic curves of CCI in predicting 1-year mortality for older adults was 0.68 (CI, 0.64-0.72).

Conclusion: There is a significant dose-response relationship in HR between CCI and 1-year mortality in Chinese older adults but involvements of functional, nutritional, and social assessments are important for comprehensive quantification of health status in older adults.

Assessment of carotid intima-media thickness in patients with axial spondyloarthritis: relationship with disease severity

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Objective: Axial spondyloarthritis (SpA) is a chronic rheumatic disease characterised by inflammation of the spine, sacroiliac and peripheral joints, causing pain and functional disabilities. As the disease advances, syndesmophytes will form in axial joints leading to further functional loss. Carotid intima-media thickness (IMT) is widely used as a surrogate marker for subclinical atherosclerosis. It is proposed that persistent systemic inflammation in SpA is associated with early carotid atherosclerosis. The aim of this study was to evaluate the changes of carotid IMT in patients with axial SpA and their relationship with the underlying disease severity.

Methods: A total of 104 patients with axial SpA (mean age, 45.5 ± 13.3 years; 69.2% male) and 52 age- and gender-matched healthy controls were enrolled into the study. All patients underwent clinical examination, laboratory blood tests, and spine radiographs. High-resolution ultrasonography was used to measure far-wall carotid IMT at the common carotid artery. Bilateral maximum carotid IMT measurements were performed offline using semi-automated imaging processing software and the mean carotid IMT was calculated for evaluating atherosclerosis. The disease duration, erythrocyte sedimentation rate and C-reactive protein levels, Bath Ankylosing spondylitis Disease Activity Index (BASDAI), and Bath Ankylosing Spondylitis Functional Index (BASFI) scores were recorded. The disease severity of the axial SpA patients was assessed by modified Stoke Ankylosing Spondylitis Spine Score (mSASSS).

Results: Carotid IMT was significantly increased in patients with axial SpA compared with controls (0.78 ± 0.19 mm vs 0.69 ± 0.10 mm; P < 0.001). In axial SpA patients, BASDAI (β = 0.22, P = 0.03), BASFI (β = 0.45, P < 0.001), and mSASSS (β = 0.60, P < 0.001) correlated significantly with carotid IMT. Multivariate analysis adjusting for potential confounding factors demonstrated that mSASSS was independently associated with carotid IMT (β = 0.23, 95% confidence interval, 0.00-0.01; P=0.03). In patients with mSASSS score above the median value (14.75), carotid IMT was significantly higher compared with patients below the median value (0.85 ± 0.18 mm vs 0.71 ± 0.16 mm; P < 0.001).

Conclusion: Our study demonstrated that patients with axial SpA had early carotid atherosclerosis. mSASSS remained independently associated with carotid IMT after adjusting for the confounding factors. Importantly, the group with mSASSS score above the median value had a significantly higher carotid IMT. In conclusion, this study shows that patients with axial SpA have a tendency to develop subclinical atherosclerosis which correlates significantly with the disease severity. Whether effective disease control could prevent the development of atherosclerosis remains to be investigated.

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Peptidyl-prolyl isomerase promotes DNA double-strand break repair through regulation of the phosphorylation of ataxia telangiectasia mutated protein

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Introduction: Ataxia telangiectasia mutated (ATM) signalling is essential for the coordination of DNA double-strand break repair through phosphorylating its downstream target for chromatin relaxation. Peptidyl-prolyl isomerase (PIN1) interacts with and modulates functions of many phosphorylated proteins that are involved in cell cycle progression and oncogenesis. In this study, we investigated the role of PIN1 in the regulation of DNA repair function of ATM.

Methods: The interaction between PIN1 and ATM was investigated by a mammalian tandem-affinity purification (TAP) system and co-immunoprecipitation (co-IP) assay. The effect of PIN1 depletion or overexpression on ATM phosphorylation was determined by western blot analysis. To determine the effect of PIN1 on DNA repair, the rate of gH2AX foci loss after irradiation was measured by immunofluorescence.

Results: Mass spectrometry analysis of the TAP system and the co-IP assay verified the interaction between PIN1 and ATM. PIN1 overexpression enhanced the phosphorylation level of ATM after irradiation. In contrast, overexpression of PIN1 mutants defective for protein binding (W34A) or for isomerase activity (S67E) did not modulate the phosphorylation level of ATM. Twenty-four hours after induction of double-strand DNA breaks by irradiation, more gH2AX foci were found in Pin1-null cells than that in wild-type cells, indicating that loss of PIN1 expression delayed DNA repair. Similarly, enforced expression of wild-type PIN1 in Pin1-null cells showed less gH2AX foci, compared with vector and PIN1 mutant (W34A) controls. In contrast, treatment with ATM inhibitor in PIN1 overexpressing cells did not reduce the rate of gH2AX loss as compared with that in PIN1 mutant (W34A) overexpressing cells, suggesting that the inhibition of ATM attenuated DNA repair facilitated by PIN1.

Conclusion: Enhanced repair of irradiation-induced DNA damage by PIN1 is mediated through modulation of the phosphorylation of ATM.

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Ablation of APPL2 in pancreatic β-cells causes defective insulin secretion

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Introduction: Our previous study demonstrated that the adaptor protein APPL1 enhances glucose-stimulated insulin secretion (GSIS), thereby alleviating diet-induced glucose intolerance in mice. In this study, we aimed to investigate whether APPL2, a close homology of APPL1, regulates β-cell functions using a β-cell specific knockout (KO) mouse model.

Methods: Glucose tolerance and insulin secretory ability were examined in APPL2 KO mice and their wild-type (Wt) littermates fed with standard chow or high-fat diet (HFD).

Results: APPL2 was abundantly expressed in pancreatic islets, and its expression was elevated in islets from obese mouse models. Genetic ablation of APPL2 causes defective GSIS, leading to glucose intolerance. Such defect was aggravated when APPL2 KO mice were under HFD feeding. Ex-vivo analysis revealed that islets lack of APPL2 displayed reduced insulin secretion in response glucose but not potassium chloride stimulation. Further analysis indicated that islets with or without APPL2 displayed similar calcium mobilisation when stimulated with glucose, ATP sensitive potassium channel blocker tolbutamide or L-arginine. These findings suggested that the defect might occur in the upstream of closure of ATP-sensitive potassium channels.

Conclusion: APPL2 is a key regulator of insulin secretion in pancreatic β-cells. However, the detailed mechanism underlying the APPL2 actions in β-cells warrants further investigation.

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Cognitive impairment in adiponectin-knockout mice

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Introduction: Alzheimer's disease (AD) is the most common cause of dementia in the elderly. AD is characterised by amyloid-beta (Aβ)–mediated neurotoxicity and neuronal insulin resistance. Adiponectin, an adipokine with insulin-sensitising and anti-inflammatory actions, bears potential as novel therapy for AD. We aimed to study the cognitive function of adiponectin-knockout (APN-KO) mice.

Methods: APN-KO and wild type (WT) mice of 9 and 18 months old had cognitive functions assessed by Morrison water maze test and open field test. Mice were sacrificed with 1 week after cognitive functions tests and forebrain cortex was studied immunohistologically for Aβ (using 2C8 and 7A1a antibodies) and microglial activation (by Iba1 immunoreactivity). Frontal cortex homogenate was analysed for insulin receptor substrate-1 phosphorylated at serine 616 (IRS-1pS^{616}, marker of insulin resistance) by western blot.

Results: APN-KO mice of 9 months old had increased anxiety (shorter distance moved, slower velocity, less time of movement, less time in centre, more time in margin and less exploration; $P < 0.05$) but indifferent in spatial memory compared to WT mice of the same age. APN-KO mice of 18 months old had increased anxiety (shorter distance moved, slower velocity, less time of movement, less time in centre, more time in margin and less exploration; $P < 0.05$) and impaired spatial memory (longer hidden platform latency; $P < 0.01$) compared to WT mice of the same age. Histologically, APN-KO mice had increased immunoreactivity for Aβ ($P < 0.0001$) and Aβ oligomers ($P < 0.0001$) and increased microglial activation ($P < 0.05$) than WT mice. Western blot revealed that frontal cortex homogenate of APN-KO mice had higher level of IRS-1pS^{616} than that of WT mice of the same age ($P < 0.05$).

Conclusion: APN-KO mice of 9 and 18 months old had impaired cognitive functions associated with increased cerebral Aβ deposition and neuronal insulin resistance compared to WT mice of the same age.

The relationship between glucose metabolism, metabolic syndrome, and bone-specific alkaline phosphatase: a structural equation modelling approach

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Introduction: Serum alkaline phosphatase plays a role in vascular calcification. It is found in various tissues, whereas bone-specific alkaline phosphatase (BAP) more specifically reflects mineral metabolism. The relationship of serum alkaline phosphatase (total and bone-specific) with diabetes and metabolic syndrome, which are two major risk factors of vascular calcification, is largely unknown. We aimed to investigate the relationships between glucose metabolism, components of metabolic syndrome (MetS), and alkaline phosphatase.

Methods: Data on 3773 non-diabetic participants of the National Health and Nutrition Examination Survey 1999–2004 were examined. Serum BAP and total alkaline phosphatase were measured as outcomes. Linear regression was used to assess the association of glucose metabolism and metabolic syndrome with serum alkaline phosphatase levels.

Results: In multivariable linear regression, HOMA2-IR ($\beta = 0.068$), HOMA2-B ($\beta = 0.081$), insulin ($\beta = 0.065$), mean arterial pressure ($\beta = 0.15$), and high density lipoprotein (HDL)–cholesterol ($\beta = 0.209$) were positively associated with BAP, whereas HOMA2-IS ($\beta = -0.065$) was negatively associated with BAP. On the other hand, only mean arterial pressure and HDL-cholesterol were significantly associated with total alkaline phosphatase. Moreover, structural equation model revealed that hypertension, low HDL, and insulin resistance had significant direct effects on serum BAP levels, whereas obesity and inflammation might have indirect effects on serum BAP levels. The overall model showed very good fit to the data (comparative fit index = 0.995, root mean square error of approximation = 0.037, and standardised root mean square residual = 0.006).

Conclusion: Glucose metabolism and MetS are significantly related to serum BAP levels. How BAP mediates vascular calcification in diabetes and MetS warrants further studies.

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Genetic variants in GREM2 are associated with bone mineral density in a southern Chinese population

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Introduction: Gremlin 2 (GREM2) is a regulator of osteoblast differentiation and osteogenesis. A recent genome-wide association study identified GREM2 as a novel susceptibility gene for trabecular volumetric bone mineral density (BMD). We investigated whether GREM2 gene variants were associated with areal BMD in southern Chinese people.

Methods: We genotyped 108 single-nucleotide polymorphisms (SNPs) in 417 cases (defined as BMD Z-score ≤ −1.28) and 359 controls (defined as BMD Z-score ≥ +1). Multivariable logistic regression using an additive model was used to evaluate the association. The most associated SNPs of BMD at the spine, femoral neck, and total hip were then replicated in an additional 454 cases and 401 controls.

Results: A total of 12, 13, and 14 SNPs showed nominal association with BMD at the spine, femoral neck, and total hip, respectively. The minor alleles of rs9728351 (odds ratio [OR] = 2.56; 95% confidence interval [CI], 1.33-4.92), rs11588607 (OR = 1.65; 95% CI, 1.14-2.4), and rs4454537 (OR = 1.87; 95% CI, 1.22-2.86) were associated with the low BMD at the spine, femoral neck, and total hip, respectively. Among these SNPs most associated with BMD, rs4454537 was successfully replicated in an independent cohort (OR = 1.59; 95% CI, 1.05-2.4). Meta-analysis showed that the minor allele of rs4454537 was associated with low total hip BMD with an OR of 1.72 (95% CI, 1.28-2.31) [$P = 3.2 \times 10^{-4}$; $P_{corrected}$=0.043).

Conclusions: The minor allele of rs4454537 is significantly associated with low BMD at the total hip of southern Chinese people. Our study further suggests GREM2 as a novel susceptibility gene for osteoporosis.

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Genetic variant in vitamin D binding protein is associated with serum 25-hydroxyvitamin D and vitamin D insufficiency in southern Chinese

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Introduction: Previous large-scale genome-wide meta-analysis identified four loci affecting 25-hydroxyvitamin D (25(OH)D) concentrations. However, whether these loci are associated with 25(OH)D concentration in southern Chinese remain unknown. Our primary aim was to examine whether the four top hits (rs2282679, rs10741657, rs12785878, and rs6013897) could be replicated in 712 southern Chinese women.

Methods: The associations between these single-nucleotide polymorphisms (SNPs), serum 25(OH)D concentration (continuous variable), and vitamin D insufficiency (dichotomised variable) were examined using multivariable linear regression and logistic regression, respectively. Age, body mass index, and season were adjusted in the model.

Results: Among these four SNPs, rs2282679 was associated with serum 25(OH)D levels ($\beta = -0.066$; $P = 9 \times 10^{-5}$) and vitamin D insufficiency (odds ratio [OR] = 1.51; 95% confidence interval [CI], 1.19-1.93; $P = 8.6 \times 10^{-4}$), whereas rs12785878 was nominally associated with vitamin D insufficiency only (OR = 0.79; 95% CI, 0.63-0.99; P = 0.042). Genotype risk score (GRS), by summing risk variants of these two SNPs, had more significant association with vitamin D insufficiency (OR = 1.38; 95% CI, 1.17-1.64; $P_{trend} = 1.76 \times 10^{-4}$) than the model that included only either SNP. The areas under receiver operating characteristic curves of rs2282679 and GRS were 0.561 (P = 0.005) and 0.576 ($P = 5 \times 10^{-4}$), respectively.

Conclusion: Our study provides an independent evidence of the associations of rs2282679 and probably rs12785878 with 25(OH)D and vitamin D insufficiency in southern Chinese.

Vitamin K intake reduces mortality in people with chronic kidney disease

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Background: Cardiovascular disease (CVD) is the leading cause of death in patients with chronic kidney disease (CKD), partly due to increased vascular calcification. Emerging evidence suggests that vitamin K plays a key role in preventing vascular calcification in CKD. However, the relationship between vitamin K intake and mortality in people with CKD remains unknown. The objective of this study was to examine the association of vitamin K intake with all-cause and CVD mortality in a nationally representative sample aged 20 years or above.

Methods: A total of 3401 participants with CKD from the Third National Health and Nutrition Examination Survey were included. Dietary intake was assessed during nutritional examination based on 24-hour dietary recall. Vitamin K intake was used in multivariate Cox regression analysis to predict all-cause and CVD mortality.

Results: During a median follow-up of 13.3 years (37 408 person-years), 1815 and 876 participants died from all-cause and CVD causes, respectively. The majority of participants had vitamin K intake lower than the recommended intake levels. In multivariable Cox-regression analysis, participants in higher quintiles (quintile 4-5) of vitamin K intake had significantly lower risk of all-cause (hazard ratio [HR] = 0.86; 95% confidence interval [CI], 0.75-1; P = 0.046) and CVD mortality (HR = 0.79; 95% CI, 0.64-96; P = 0.021) when compared with quintiles 1 through 3. Participants with vitamin K intake higher than recommended adequate intake value for vitamin K were associated with lower risk of all-cause and CVD mortality.

Conclusion: These findings suggest that adequate-to-high level of vitamin K intake reduces CVD and all-cause mortality in people with CKD.

Adiponectin gene variant +276G>T independently predicts incident coronary heart disease in men: a 16-year prospective study

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Introduction: Adiponectin has been suggested to play a protective role in the development of coronary heart disease (CHD). However, recent prospective studies suggested that high adiponectin levels are associated with a higher risk of cardiovascular mortality in an established CHD cohort. Study of genetic variants of the adiponectin gene (*ADIPOQ*) may provide more insights into the primary role of adiponectin in CHD development. Our objective was to examine the prospective relationship between the genetic variants of *ADIPOQ* and incident CHD in a 16-year prospective population-based cohort of southern Chinese.

Methods: Nine *ADIPOQ* genetic variants with potential functional relevance or shown to be associated with adiponectin levels and/or CHD were genotyped in 2196 subjects from the Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS), who were free of CHD at baseline. Among these subjects, 184 had developed CHD over the 16-year follow-up period.

Results: The *ADIPOQ* +276G>T variant was found to be independently associated with incident CHD in men but not in women, even after adjustments for different sets of conventional cardiovascular risk factors ($P_{adjusted}$ = 5.5 x 10^{-3} to 0.023; hazard ratio = 1.39 to 1.54). Moreover, the T allele of +276G>T was found to be significantly associated with reduced plasma adiponectin level (P = 0.027) in 1676 subjects with available plasma samples for analysis.

Conclusion: This study supports a protective role of adiponectin in the development of CHD in the general population.

Short-term outcomes and impact of anticoagulation reversal on warfarin-associated intracranial haemorrhage

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Introduction: Intracranial haemorrhage (ICH) is the most dreadful complication associated with warfarin use. While antidotes such as fresh frozen plasma (FFP) and vitamin K are available, its impacts on outcomes of warfarin-associated ICH remains uncertain. This study aimed to investigate the utility of anticoagulation-reversal agents in ICH patients and the impact on outcomes.

Methods: This was a single-centre registry based on the clinical outcomes of patients with warfarin-associated ICH during the period from year 1997 to 2013. Data pertaining to the index ICH, demographics, cardiovascular risk factors, anticoagulation intensity were abstracted from ePR and medical charts. Descriptive statistics were calculated and reported. Kaplan-Meier estimate was used to compare between groups of patients receiving different treatments for warfarin overdose. Multivariate analysis was performed to look for predictors of mortality.

Results: A total of 85 patients with warfarin-associated ICH (median age, 72 years; 49.4% males) were included. On admission, a majority of patients (55.3%) had international normalised ratio (INR) of ≤3.0, and only 20% had a high INR of >4. The overall 30-day mortality was 38.8%. Kaplan Meier analysis revealed that patients receiving neither FFP nor vitamin K during the admission predicted a higher 30-day mortality (P = 0.004), but there was no difference between the groups receiving FFP in combination with vitamin K, FFP alone and vitamin K alone. Further analysis using available data did not yield any other significant prognostic indicators.

Conclusion: The majority of warfarin-associated ICH occurred in patients without supratherapeutic INR (<3), and the mortality of warfarin-associated ICH remained high albeit with available antidotes.

Feasibility of implementing advance directive in Hong Kong Chinese elderly people

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Objectives: To assess the feasibility of advance directive (AD) engagement among elderly people and to explore the contributing factors associated with such engagement.

Methods: Patients admitted to the geriatric ward of Grantham Hospital, from August 2012 to June 2013, were included. Subjects who were aged 65 years or above, without dementia nor delirium, with Mini-Mental State Examination (MMSE) scores of 20 or above, and physically fit were invited to participate in the advance care planning. Subjects and their family members were interviewed by a geriatrician. Their knowledge of AD was assessed. The concept of AD was introduced and promoted. The model form of AD from Hospital Authority was used. Those who engaged in AD were compared to those who declined to find out the factors associated with engagement. The reasons for engagement were also analysed.

Results: A total of 33 patients had made a decision. Their mean age was 82.4 years; 12 (36.4%) agreed to engage in AD while 21 (63.6%) declined. There were no statistical difference in age, gender, education, MMSE, religious belief, mobility and functional state, Charlson comorbidity score, and proportion of cancer among two groups. However, elderly patients who engaged in AD were more likely to be living alone, single / widowed / separated / divorced, and to perceive their health as poor or very poor. On the other hand, those who declined engagement were more likely to have children and to have either spouse or children as their main caregivers. The main reasons for enacting AD included "to avoid suffering" (66.7%), "to avoid burden to family members" (33.3%), "quality of life is more important than length of life" (25%), and "past experience from friends or others" (25%). The main reasons for declining AD included "family will decide for them" (71.4%), "not ready to discuss it" (28.6%), and "let nature to decide for them" (23.8%).

Conclusion: It is feasible to implement AD among elderly people if suitably promoted. Social and family conditions are found to be important factors in determining the engagement of AD.

Neuroprotective effects of melatonin and calpeptin in a rat model of focal cerebral ischemia

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Introduction: Melatonin is a potent-free radical scavenger and antioxidant. Previously, we have demonstrated beneficial effects of pretreatment with melatonin in mild and severe focal cerebral ischaemia in rodent models. Cerebral ischaemia increases intracellular Ca^{2+} concentrations, and activates several calcium-dependent proteases including calpain. Pretreatment with calpeptin, a novel calpain inhibitor, has been reported to reduce the cerebral infarct volume. In addition, it also decreases the neuronal apoptosis in hippocampal CA1 sector and improves the behavioural deficit in a rat stroke model. The aim of this study was to investigate the neuroprotective role of a post-ischaemia treatment with melatonin and / or calpeptin, and whether combining the two exerts synergistic or addictive effects in transient focal ischaemic stroke in rats.

Methods: Male Sprague-Dawley rats (6-8 weeks) were anaesthetised with sodium pentobarbital to undergo right-sided endovascular middle cerebral artery occlusion (MCAO) for 90 minutes followed by 24 hours of reperfusion before being sacrificed. A single or a combined dose of melatonin (50 μg/kg) and / or calpeptin (50 μg/kg) were given via an intracerebroventricular injection at 10-15 minutes after onset of the reperfusion. Sham group with injection of vehicle only was used as a control group. Neurological behaviour was assessed using Neurological Deficit Scoring System (NDSS) test and cerebral infarction volumes were evaluated by TTC-staining.

Results: Infarction volumes and NDSS scores were lower in the calpeptin group but not in melatonin group when compared with the control. The combining effects of melatonin and calpeptin will be studied further.

Conclusion: Our results suggest that post-ischaemia treatment with calpeptin but not melatonin at 50 μg/kg protects against focal MCAO model in rats.

Sorafenib combined with azacitidine is an effective post-remission therapy for FLT-ITD+ acute myeloid leukemia

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Introduction: Fms-like tyrosine kinase 3 (FLT3) internal tandem duplication (ITD) occurs in 30% of patients with acute myeloid leukaemia (AML) and confers a poor prognosis.

Methods: A total of 23 patients with FLT-ITD+ AML were treated with sorafenib (200-400 mg twice daily). Of them, 22 patients achieved clearance of marrow blasts at a median of 25 days (range, 19-91 days); 13 patients received sorafenib single-agent as post-remission therapy, 6 patients received sorafenib combined with azacitidine, and 3 patients received sorafenib combined with high-dose cytarabine (HDAC) consolidation. To validate the clinical observation, synergism between sorafenib and azacitidine was evaluated in 2 FLT-ITD cell lines MOLM-13 and MV4-11. Leukaemic cells treated with sorafenib, azacitdine and in-combination were evaluated for cell viability and apoptosis, differentiation, and leukaemia-initiating activity by xenotransplantation.

Results: With a median follow-up of 182 (range, 61-694) days, the median progression-free survival (PFS) of the entire cohort was 71 days (95% confidence interval [CI], 59.7-82.3) and the median overall survival (OS) was 198 days (95% CI, 155-241). Nine patients who received combination post-remission therapy had a significantly better PFS (median 118 days vs 63 days, P = 0.008) and a trend towards better OS (median 227 days vs 182 days, P = 0.07). A favourable PFS was preserved when patients given HDAC were excluded (median 116 days with sorafenib plus azacitidine vs 63 days with sorafenib monotherapy, P = 0.05). In MOLM-13 and MV4-11 cell lines, treatment with combined sorafenib and azacitidine showed additive effect in terms of cell death and apoptosis. There was a trend towards enhanced myeloid differentiation with combination treatment. Sublethally irradiated anti-CD122 primed NOD/SCID mice transplanted with leukaemic cells treated with combined sorafenib and azacitidine showed remarkable suppression of leukaemic engraftment potential.

Conclusion: Information from this study suggests that sorafenib when combined with azacitidine is effective in prolonging remission in these patients and bridge these patients to definitive treatment like allogeneic haematopoietic stem cell transplantation.

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Oral arsenic trioxide–based maintenance without haematopoietic stem cell transplantation at second remission in acute promyelocytic leukaemia: a prospective follow-up study of 65 patients

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Introduction: Approximately 20% of patients with acute promyelocytic leukaemia (APL) relapse after initial remission. More than 80% of these patients achieve a second complete remission (CR2). Autologous haematopoietic stem cell transplantation (HSCT) at CR2 is a widely accepted post-remission strategy. However, only a few prospective trials have examined the optimal post-remission therapy for relapsed APL.

Methods: We prospectively evaluated the outcome of 65 patients with APL in their first relapse (R1) treated with oral arsenic trioxide (As_2O_3) and chemotherapy-based re-induction followed by oral As_2O_3–based maintenance. A total of 65 patients (37 male and 28 female) at a median age of 39 (3-76) years were treated with oral As_2O_3, all-trans-retinoic acid, and ascorbic acid combined with idarubicin for re-induction. On achieving CR2, two cycles of idarubicin consolidation were given. That was followed by oral As_2O_3–based maintenance for 2 years.

Results: The median duration of follow-up was 90 (21-378) months. All evaluable patients achieved CR2. Twenty-one patients (32.8%) subsequently had a second relapse (R2). On multivariate analysis, male gender was associated with an increased risk of R2 ($P = 0.01$). The age, white blood cell (WBC) count, platelet count, peak WBC during re-induction and the occurrence of differentiation syndrome did not impact on the risk of R2. The median overall survival (OS) was not reached. The 5-year and 10-year OS were 82.2% and 74.4%, respectively. Significantly inferior OS was associated with male gender ($P = 0.02$), prior oral As_2O_3–based maintenance at CR1 ($P = 0.01$) and central nervous system involvement at relapse ($P < 0.001$). The median leukaemia-free survival (LFS) after CR2 was not reached. The 2-year and 5-year LFS were 72.4% and 64.1%, respectively. Male gender and relapse from prior oral As_2O_3–based maintenance at CR1 were associated with worse LFS ($P = 0.01$ and $P = 0.04$, respectively).

Conclusion: Our study showed that durable remissions and long-term survival were achieved with oral As_2O_3–based maintenance without the need of autologous HSCT at CR2.

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Valganciclovir prophylaxis prevented Epstein-Barr virus reactivation during alemtuzumab therapy

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Introduction: Primary infection with Epstein-Barr virus (EBV) usually occurs in childhood. EBV subsequently establishes latency in B-cells. In immunocompetent hosts, EBV reactivation is prevented by virus-specific CD8+ cytotoxic T-cells and CD4+ T-cells. Impairment of cellular immunity predisposes to EBV reactivation and EBV-related lymphomas. Alemtuzumab is a humanised monoclonal antibody against CD52 expressed on all B and T lymphocytes. Alemtuzumab treatment leads to profound and protracted T-cell depletion, increasing the risk of reactivation of latent herpes viruses, including cytomegalovirus and EBV.

Methods: All consecutive patients treated between March 2005 and June 2013 with alemtuzumab (30 mg/day, thrice weekly) were studied. Standard antiviral prophylaxis included valganciclovir (900 mg/day, thrice weekly) continued throughout and for 12 weeks after completion of treatment. Plasma EBV DNA was quantified serially by quantitative polymerase chain reaction targeting the *EBNA1* gene.

Results: A total of 258 samples were quantified in 29 patients, at a median of 7 (3-25) specimens per patient. Twenty-four patients never had any quantifiable plasma EBV DNA. Five patients (17%) developed EBV reactivation, at a media of 64 (14-273) days after alemtuzumab treatment, for a median duration of 6 (1-12) weeks. The median peak EBV DNA level was 51 (9.7-557) x 10^2 IU/mL. There was no statistically significant association between EBV reactivation with age, gender, duration and cumulative dose of alemtuzumab, and nadir lymphocyte count. For cases with EBV reactivation, two patients showed low levels of about 10 x 10^2 IU/mL, one log less than that typically found in EBV-associated lymphomas in immunocompetent patients, and several logs less than those found in post-transplant lymphoproliferative disease (PTLD). In the remaining three patients, who had received two courses of alemtuzumab, EBV reactivated at higher levels (>50 x 10^2 IU/mL), comparable with what might be found in EBV-associated lymphomas in immunocompetent patients, but still 1-2 logs lower than that in PTLD.

Conclusion: Our findings suggest that valganciclovir may be useful as a prophylaxis to prevent EBV reactivation in high-risk populations, including solid organ and haematopoietic stem cell transplant recipients. This proposition should be tested prospectively in future studies.

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Oral arsenic trioxide–based regimen as salvage treatment for relapsed or refractory mantle cell lymphoma: a prospective study

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Introduction: Mantle cell lymphoma (MCL) is aggressive, and relapsed / refractory disease has a poor outlook. In patients with relapsed / refractory MCL, optimal treatment strategy remains undefined. Oral arsenic trioxide (As_2O_3) was initially developed for the treatment of relapsed acute promyelocytic leukaemia. As_2O_3 inhibits neoplastic cellular proliferation by a wide array of mechanisms, including induction of apoptosis, targeting of signalling pathways, and down-regulation of BCL-2. Evidence in vitro also suggested that As_2O_3 might be effective in lymphoma, but clinical data are hitherto not available.

Methods: In this study, we investigated the use of an oral-As_2O_3–based regimen for the treatment of patients with relapsed / refractory MCL. Thirty-nine patients (men, 34; women, 5) with a mean age of 64 (41-82) years with relapsed / refractory MCL, who had received 2 (1-5) prior regimens and were ineligible for high-dose chemotherapy, were treated with a continuous oral regimen, comprising oral-As_2O_3, chlorambucil, and ascorbic acid.

Results: The overall response rate was 49% (complete response, 28%; partial response, 21%). Only grade 1/2 toxicities were observed (haematologic, 56%; hepatic, 8%). Independent prognostic factors for response were increased lactate dehydrogenase ($P = 0.04$) and unfavourable MCL international prognostic index ($P = 0.04$). At a median follow-up of 21 (1-118) months, the median progression-free survival (PFS) was 16 months, and overall survival (OS) was 38 months. The 2-year and 5-year PFS were 41% and 29%, respectively. The 2-year and 5-year OS were 56% and 43%, respectively. Independent prognostic factors for PFS were female gender ($P = 0.002$), Eastern Cooperative Oncology Group (ECOG) performance score of 2 ($P = 0.009$), and non-response to treatment ($P < 0.001$). Independent prognostic factors for OS were female gender ($P < 0.001$), ECOG performance score of 2 ($P = 0.03$), non-response to treatment ($P < 0.001$), and disease progression while on treatment ($P = 0.05$).

Conclusion: These findings showed that an oral regimen of oral-As_2O_3, chlorambucil, and ascorbic acid was an active regimen with minimal toxicity in relapsed / refractory MCL, achieving durable responses in some cases.

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Dysfunctional perivascular adipose tissue impairs endothelial function via production of superoxide in obese mice

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Introduction: Endothelial dysfunction (ED) is prominent in obesity, but the precise mechanisms are not well understood. Perivascular adipose tissue (PVAT) produces vasoactive substances that regulate vascular tone. The aim of this study was to investigate whether obesity induces ED via PVAT and to elucidate the underlying mechanisms.

Methods: Six-week-old C57BL/6J mice were fed with either standard chow or high-fat diet for 12 weeks. The obese mice were exposed to cold environment (4°C) for 6 days. The aortic rings in the presence or absence of PVAT were isolated and the endothelium-dependent relaxation in response to acetylcholine was measured by wire myograph. The superoxide production in PVAT was determined by DHE staining and lucigenin assay.

Results: Endothelium-dependent relaxation was reduced in the presence of PVAT, demonstrating that PVAT elicits an anti-relaxation effect to blood vessel. In the absence of PVAT, aortic rings did not show any difference in endothelium-dependent vasodilatation between lean and obese mice. However, in the presence of PVAT, the relaxation was significantly impaired in aortic arteries from obese mice, suggesting that obesity potentiates anti-relaxation activity of PVAT. PVAT from obese mice exhibited increased superoxide production, whereas treatment with the superoxide scavenger Tiron partially rescued impaired endothelial function. Interestingly, cold exposure (4°C) decreased obesity-induced superoxide concentration in PVAT, leading to improved vasodilatation.

Conclusions: Obesity-induced ED of aortic arteries is largely mediated by its surrounding PVAT, which produces superoxide to counteract vasodilatation.

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A novel role of ADP-ribosylation factor like 4aa(ARL4aa) in zebrafish haematopoiesis

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Introduction: Arl4aa is the member of ADP-ribosylation factor family, a group of GTP-binding proteins controlling membrane proteins trafficking and distribution of intracellular proteins. Previous studies showed that *arl4aa* expression was up-regulated in a zebrafish chordin mutant in which haematopoiesis was expanded. However, its role has not been determined. In this study, we characterised the tempo-spatial expression pattern and functional role of *arl4aa* in haematopoiesis in zebrafish.

Methods: Gene expression of arl4aa and other haematopoietic genes were evaluated by both whole-mount in-situ hybridisation and quantitative real-time polymerase chain reaction. Knock-down (KD) of arl4aa was achieved by morpholinos (MO). Comparison of data was evaluated by Students' unpaired *t*-test.

Results: arl4aa was expressed in the intermediate cell mass at 18 hpf, along the dorsal aorta (DA) at 36 hpf and the caudal haematopoietic tissue (CHT) at 48 hpf. KD of arl4aa resulted in reduction of c-myb expression (a marker of definitive hematopoietic stem cells [HSC]) in the DA and CHT at 36 and 48 hpf and rag1 expression in the thymus at 96 hpf (a marker of T-lymphoid development that arises from definitive HSC). Defective gene expression could be rescued by arl4aa mRNA that was resistant to the MO, attesting to the specificity of the haematopoietic phenotype. Primitive macrophage development was increased, as shown by increased l-plastin and mpeg1 expression over the yolk sac at 24 hpf. Genes associated with primitive HSC (lmo2, scl) and erythropoiesis (gata1, embryonic haemoglobins) were not affected.

Conclusion: We demonstrated a hitherto undescribed function of arl4aa during embryonic haematopoiesis in regulating both primitive macrophage and definitive HSC development. The mechanisms of action are currently being evaluated.

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Relative efficacies of dronedarone and class IC anti-arrhythmic drugs in rhythm control of atrial fibrillation

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Introduction: Maintaining sinus rhythm in patients with non-permanent atrial fibrillation (AF) is a clinical challenge. Dronedarone, a class III anti-arrhythmic drug, has expanded the limited repertoire of rhythm control agent in AF. Its benefits on clinical outcomes were proven in the ATHENA trial. Yet little is known on the relative efficacies in rhythm control between dronedarone and other anti-arrhythmic drugs. This study aimed to compare AF-free survival among patients treated with dronedarone, flecainide, or propafenone.

Methods: Patients dispensed with dronedarone, flecainide, or propafenone for the first time in Queen Mary Hospital from September 2008 to August 2013 were identified via the Hospital Authority database. Those having non-permanent AF were included. Those aged less than 45 years were excluded. The primary endpoint was the discontinuation of the drug of interest within the first year, a surrogate marker of breakthrough AF. Survival data were analysed by Kaplan-Meier estimate; unadjusted AF-free survival was compared between the three groups. Other clinical information, such as age at prescription, length of AF history before prescription, past health, concurrent medications, and previous anti-arrhythmic drugs used were included in a univariable regression by Cox's proportional hazards model. Multivariable regression ensued to adjust for confounders.

Results: A total of 303 patients (mean age, 66 years) were included in the analysis. The mean duration of AF prior to the anti-arrhythmic drugs was 54 months. Baseline demographics were largely homogeneous across the three groups. The mean time to AF recurrence was 4.4 months. Unadjusted AF-free survival between the three groups did not differ significantly (log-rank $P < 0.11$). Multivariable Cox's regression confirmed the lack of difference in AF-free survival between the three groups (flecainide $P < 0.230$, propafenone $P < 0.223$).

Conclusion: Clinical data show that dronedarone is not inferior to class IC anti-arrhythmic drugs in maintaining sinus rhythm for patients with non-permanent AF. On top of the improved clinical outcomes in ATHENA, this study adds further evidence to support the use of dronedarone as rhythm control agent in AF.

Dabigatran discontinuation in 'real-world' practice in Hong Kong

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Introduction: Dabigatran is an oral direct thrombin inhibitor for stroke prophylaxis in patients with non-valvular atrial fibrillation (AF). While serving as an appealing alternative to warfarin, dabigatran remains a self-financing item in Hospital Authority in Hong Kong. As a result, the tolerability and long-term financial sustainability of the patients may affect its adherence. The aim of this study was to describe the dabigatran discontinuation rate and possible predictors in real-world practice in Hong Kong.

Methods: This was a single-centred observational study. Patients with non-valvular AF receiving dabigatran for stroke prevention were identified from our hospital registry of AF. Data pertaining to AF, demographics, cardiovascular risk factors, and medications were entered into the Clinical Management System Database. The primary endpoint was dabigatran discontinuation during the follow-up period. Reasons of discontinuation were retrieved from the medical records and discharge summaries from our hospital as well as other institutions.

Results: A total of 410 patients were identified. The mean age was 72 years with a male predominance (56.6%). The mean follow-up duration of dabigatran was 16 months. Of 410 patients, 95 (23.2%) permanently discontinued dabigatran with the mean time-to-discontinuation of 8 months. A majority of discontinuation (47%) was due to patient-driven factors such as financial concerns. The second most common reason was dyspepsia (19%). Dabigatran discontinuation was significantly associated with lower estimated glomerular filtration rate, congestive heart failure at baseline, prior use of proton-pump inhibitor and antacid as well as concurrent use of proton-pump inhibitor and amiodarone.

Conclusion: Dabigatran discontinuation is common among Chinese AF patients. Our observations highlight the importance of patient selection, doctor-patient communication before the initiation of a possible life-long, self-financing medication.

Whole-exome sequencing identified a novel mutation in *PMCA4* gene for autosomal dominant familial spastic paraplegia

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Introduction: Familial spastic paraplegia (FSP) is a heterogeneous group of neurodegenerative disorder; some of them are due to unknown causes. This study aimed to elucidate the genetic cause of autosomal dominant FSP.

Methods: DNA from 4 symptomatic and 2 asymptomatic of two-generation Chinese kindred with FSP was analysed by whole-exome sequencing. Reads mapping and variants calling were performed. Single nucleotide and short insertion-deletion variants were prioritised using KGGSeq. Variants whose gene products have protein-protein interaction causing various forms of FSP were prioritised. We replicated the short list of candidate genes by conventional Sanger sequencing with all available family members, and with whole-exome sequences from 1000 healthy Chinese controls to exclude false positives. Effects on cytosolic Ca^{2+} flux were evaluated by confocal microscopy in Ca^{2+}-sensitive fura-2-stained human neuroblastoma SH-SY5Y cells. Protein-folding properties were evaluated using AGGRESCAN.

Results: We identified a novel missense mutation (c.803G>A, p.R268Q) in *PMCA4* gene. Co-segregation with phenotype and validation were confirmed by Sanger sequencing. Computational modelling suggested that this mutation might affect protein folding as predicted by higher folding free energy. Stable overexpression of R268Q mutant protein in SH-SY5Y cells significantly delay amelioration of depolarisation-induced Ca^{2+} overload compared with cells overexpressing wild-type protein.

Conclusions: Plasma membrane Ca^{2+}-ATPase isoform 4 (PMCA4; ATP2B4) has not been associated with FSP. PMCAs are important in Ca^{2+} homeostasis by removing excess intracellular calcium. Of the four isoforms, PMCA4 is ubiquitously expressed, and is localised to lipid rafts found in neuronal dendrites containing various postsynaptic protein complexes, suggesting a signalling role at synaptic nerve terminals. Our findings indicate that *PMCA4* mutation may perturb neuronal calcium balance, leading to calcium overload, excitotoxicity, and neurodegeneration.

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Trend of endovascular angioplasty and stenting in treating extracranial cerebrovascular steno-occlusive disease

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Introduction: Endovascular angioplasty and stenting for extracranial cerebrovascular disease is gaining popularity in recent years.

Methods: We retrospectively reviewed all extracranial cerebrovascular angioplasty procedures performed by the neurovascular team in our centre.

Results: From year 2005 to 2013, there were 71 extracranial cerebrovascular procedures performed in 63 patients (16 female; mean age, 67.5 years; range, 39-89 years), with 81 vessels treated. The number of procedure performed increased readily in recent years, with only 23 procedures done between 2005 and 2009, increased to 15 in 2010-11, and to 33 in 2012-13. The most common procedure performed was carotid artery stenting (CAS), with a total of 61 vessels stented. There was 15 vertebral artery stenting and 5 subclavian artery stenting. In nine procedures, multiple vessels stenting were performed in same session. All procedures were performed successfully, except in one case the procedure was terminated when the distal internal cerebral artery was found to be total occluded intra-operatively. There was no peri-operative transient ischaemic attack, stroke, myocardial infarction, or death. There were only one major peri-operative complication encountered. It was a transient period of profound bradycardia with hypotension shortly after bilateral CAS. There was one case of delayed hyper-perfusion syndrome. Both cases recovered completely without deficit. There were four cases of in-stent restenosis. All but one restenosis were asymptomatic.

Conclusion: Endovascular intervention had gained popularity for treatment of extracranial cerebrovascular stenotic disease. The procedure was of low risk (2.8%) with a high success rate. The restenosis rate was low (6.3%).

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Adipocyte fatty acid–binding protein potentiates toxic lipids-induced endoplasmic reticulum stress via suppression of JAK2-dependent autophagy

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Introduction: Chronic inflammation is the key link between obesity and its related metabolic complications. Endoplasmic reticulum (ER) stress is the potent trigger of inflammation in obese adipose tissue but how ER stress in immune cells relates to inflammation is unclear. Adipocyte fatty acid–binding protein (A-FABP) regulates endotoxin-induced inflammation in macrophages by forming a positive feedback loop with c-Jun-N terminal kinase (JNK) which is the downstream regulator of ER stress. Defective autophagy is shown in obese liver which leads to insulin resistance and elevated ER stress. Here we investigate the role of A-FABP in association with autophagy in potentiating toxic lipids-induced ER stress in macrophages.

Methods: RAW264.7 macrophages infected with adenovirus-overexpressing A-FABP or luciferase or pre-treated with or without A-FABP inhibitor BMS3009403, and primary macrophages derived from A-FABP knockout mice or their wild-type littermates were treated with palmitic acid (PA) or vehicle. RAW264.7 macrophages were transfected with siRNA of autophagic protein Atg7 or scramble RNA followed by the stimulation of PA. Macrophages were treated with PA in the presence or absence of JAK2 inhibitor AG490. The autophagic flux, mRNA and protein expression of ER stress markers, autophagic proteins and inflammatory markers were determined by real-time quantitative polymerase chain reaction and western blot analysis.

Results: Over-expression of A-FABP potentiates PA-induced ER stress and inflammatory cytokine expression while BMS309403 pre-treatment reverses the condition. PA-induced ER stress was alleviated in RAW 264.7 macrophages treated with BMS309403 and A-FABP deficient macrophages and was accompanied by enhanced autophagic flux comparing to their relative controls. Suppression of autophagy in macrophages by knocking down the autophagic protein Atg7 enhanced PA-induced ER stress and inflammatory cytokine in macrophages. Treatment of AG490 reduced the autophagic protein expression while further enhanced the ER stress in response to PA stimulation. PA-induced activation of JAK2 was attenuated in the presence of A-FABP.

Conclusion: A-FABP potentiates toxic lipids-induced ER stress through inhibition of JNK-dependent autophagy macrophages.

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Lipocalin 13 protects mice against diet-induced insulin resistance potentially by lowering intramuscular lipid content in skeletal muscle

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Introduction: Lipocalin 13 (LCN13) is recently identified as a metabolic hormone that can improve glucose metabolism and ameliorate hepatic steatosis in diet-induced obese mice. However, the molecular mechanism underlying LCN13 action is still unclear. In addition, LCN13 is highly expressed in skeletal muscle; up to date, there is no study of LCN13 action in this metabolically active tissue.

Methods: We first raised specific antibody against mouse LCN13 and developed ELISA assay to measure circulating LCN13 levels in mice. Secondly, we generated the adenovirus gene delivery system to over-express LCN13 in mice and performed a comprehensive metabolic characterisation of mice with LCN13 over-expression.

Results: We found that diet-induced obesity (DIO) was associated with reduced circulating LCN13 levels in mice. Over-expression of LCN13 could ameliorate glucose intolerance and insulin resistance in DIO mice. Interestingly, the intramyocellular lipid content was significantly lower and the insulin sensitivity in skeletal muscle was improved.

Conclusion: We established the muscle as a novel target of LCN13 action. Our data indicate that LCN13 promotes insulin action in skeletal muscle by lowering intramuscular lipid content, and therefore attenuates diet-induced insulin resistance in mice.

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Adipocyte SIRT1 protects against obesity-induced endothelial dysfunction via enhancing brown-remodelling of perivascular adipose tissue in mice

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Introduction: SIRT1 is a class III, NAD-dependent histone that plays a key role in controlling adipocyte phenotypes and metabolism. In health, perivascular adipose tissue (PVAT) exhibits brown adipose features and its dysfunction is implicated in cardiovascular diseases. In the present study, we aimed to investigate the role of SIRT1 within PVAT in modulating obesity-evoked endothelial dysfunction.

Methods: Wild-type (WT) and adipocyte-specific SIRT1 knockout mice (AKO) were fed with standard chow or westernised diet for 12 weeks. The aortic rings with or without PVAT were subjected to wire myograph to assess endothelium-dependent relaxation (EDR). Expression of the brown adipocyte markers UCP-1 and PGC1α as well as adiponectin were evaluated by Western blotting, immunohistochemistry and / or quantitative polymerase chain reaction. DHE staining and lucigenin assay were used to measure superoxide levels.

Results: In the presence of PVAT, the EDR was significantly impaired in aorta from obese mice, and such an impairment was further exacerbated in obese AKO mice. PVAT in lean WT mice displayed a brown phenotype, whereas SirT1 deficiency augmented obesity-induced brown-to-white transition. In WT mice, chronic cold exposure (4°C for 1 week) reversed obesity-induced attenuation of brown phenotypes, thereby leading to improved vascular reactivity by reducing superoxide and increasing adiponectin. However, all these beneficial effects of chronic cold exposure were abrogated in AKO mice.

Conclusion: The brown phenotype of PVAT is associated with increased endothelial functions in blood vessels. SIRT1 plays a pivotal role in controlling PVAT browning, which in turn causes decreased superoxide production and increased adiponectin to protect vascular injury.

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Hyperimmune intravenous immunoglobulin treatment: a multicentre double-blind randomised controlled trial for patients with severe A(H1N1)pdm09 Infection

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Background: Experience from influenza pandemics suggested that convalescent plasma treatment given within 4 to 5 days of symptom onset might be beneficial. However, robust treatment data are lacking.

Methods: This was a multicentre prospective double-blind randomised controlled trial. Convalescent plasma from patients who recovered from the 2009 pandemic influenza [A(H1N1)pdm09] infection was fractionated to hyperimmune intravenous immunoglobulin (H-IVIG) by CSL Biotherapies, Australia. Patients with severe A(H1N1)pdm09 infection on standard antiviral treatment requiring intensive care and ventilatory support were randomised to receive H-IVIG or normal IVIG manufactured before 2009 as control. Clinical outcome and adverse effects were compared.

Results: Between 2010 and 2011, 35 patients were randomised to receive H-IVIG (17 patients) or IVIG (18 patients). One defaulted patient was excluded from analysis. No adverse event related to treatment was reported. Baseline demographics and viral load before treatment were similar between the two groups. Serial respiratory viral load demonstrated that H-IVIG treatment was associated with significantly lower day 5 and 7 post-treatment viral load when compared to the control ($P = 0.04$ and $P = 0.02$, respectively). The initial serum cytokine level was significantly higher in the H-IVIG group but fell to similar level 3 days after treatment. Subgroup multivariate analysis of the 22 patients who received treatment within 5 days of symptom onset demonstrated that H-IVIG treatment was the only factor which independently reduced mortality (odds ratio = 0.14; 95% confidence interval, 0.02-0.92; $P = 0.04$).

Conclusions: Treatment of severe A(H1N1)pdm09 infection with H-IVIG within 5 days of symptom onset was associated with a lower viral load and reduced mortality.

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Decorin inhibits cytokine-induced epithelial-to-mesenchymal transition in mesothelial cells through inhibition of p38 MAPK phosphorylation

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Introduction: Peritoneal dialysis (PD) is an established renal-replacement therapy for patients with end-stage renal failure. Frequent episodes of peritonitis during PD result in peritoneal fibrosis, which can lead to the cessation of PD. Mesothelial cells line the peritoneal cavity and play a pivotal role in peritoneal fibrosis since they can undergo epithelial-to-mesenchymal transition (EMT) and contribute to matrix protein synthesis. We previously found that mesothelial cells synthesise decorin, a dermatan sulfate proteoglycan with anti-fibrotic properties. In the present study, we investigated whether decorin could regulate EMT in human mesothelial cells following their exposure to cytokines.

Methods: Confluent, growth-arrested mesothelial cells were stimulated with exogenous TGF-beta 1 and IL-1beta (10 ng/mL for both) for 24 hours. These cytokines were increased in the peritoneal cavity of PD patients during episodes of peritonitis. In parallel studies, cells were incubated with specific inhibitors of ERK, p38 MAPK and JNK (PD98059, SB203580 and SP600125 respectively), or exogenous decorin (0-1000 ng/mL) for 1 hour before stimulation. Expression of E-cadherin, snail and fibronectin, and phosphorylation of ERK, p38 MAPK and JNK was determined by Western blot analysis.

Results: Exogenous TGF-beta1 and IL-1beta induced EMT in mesothelial cells, decreased E-cadherin expression, and increased snail and fibronectin synthesis. Incubation of cells with PD98059, SB203580, and SP600125 showed that the MAPK signalling pathways contributed to the induction of EMT and fibrogenesis in mesothelial cells. Incubation of cells with decorin-inhibited EMT and fibronectin synthesis induced by TGF-beta1 and IL-1beta ($P < 0.05$, for all). The effect of decorin was mediated through the suppression of p38 MAPK phosphorylation. Gene silencing of decorin using RNAi resulted in the amplification of TGF-induced fibronectin.

Conclusions: Our data demonstrate that decorin can suppress cytokine-induced EMT in mesothelial cells through modulation of the p38 MAPK signalling pathway.

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Relative versus absolute drug-therapy benefits and safety: Lessons from ARISTOTLE

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Introduction: ARISTOTLE was a double-blind, double-dummy, randomised clinical trial in patients with atrial fibrillation and one other risk factor for stroke, and compared oral anticoagulation with warfarin (targeted to achieve an international normalised ratio [INR] of 2.0 to 3.0) versus apixaban (5 mg x 2 daily).[1] Whilst apixaban conferred significantly greater efficacy (stroke/embolic event prevention) and was safer (less frequent bleeding), consideration of these issues in relative terms can be uninformative. To appreciate the overall cost-effectiveness of such treatments, absolute benefits should be considered.

Methods & Results: As previously described,[2] we therefore derived unadjusted estimates of relative risk (RR) and number needed to treat (NNT)/year values for prophylaxis with apixaban compared to warfarin for principal efficacy and safety endpoints published in ARISTOTLE (Table).

No. of patients (median follow-up)	Pre-specified endpoint	%RR (95% confidence interval)	NNT/year (95% confidence interval)
Apixaban - 9120	Stroke / systemic embolism	80 (66 to 96)	303 (169 to 1501)
Warfarin - 9003	Haemorrhagic stroke	51 (35 to 75)	428 (273 to 987)
(1.8 years)	Death from any cause	90 (80 to 100)	238 (119 to ∞)

Discussion & Conclusion: Whilst the favourable RR values pertaining to apixaban for stroke / systemic embolism, haemorrhagic stroke, and death seem striking, in absolute terms (NNT/year) the benefits appear less impressive. Moreover, treatment with the newer oral anticoagulants is currently much more costly than using warfarin, though the long-term impact of embolic events and major bleeds are both financially and emotionally devastating. Thus, when allocating resources for thromboembolic prophylaxis in patients with atrial fibrillation, the meagre absolute number of additional events prevented with apixaban must be weighed against the consequential long-term accrued savings as well as those from avoidance of INR monitoring.

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The effects of cigarette smoke on lipopolysaccharide-mediated inflammatory responses in airway epithelial cells

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Introduction: Chronic obstructive pulmonary disease (COPD) is a highly prevalent disease. Cigarette smoke is the major cause by stimulating the production of inflammatory chemokines, such as interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1), causing chronic inflammation in the airways. However, cigarette smoking might induce an immunosuppressive effect upon lung infection by bacteria, such as *Pseudomonas aeruginosa* during acute exacerbation in COPD, leading to bacteria colonisation in the airways and further chronic inflammation in the airway of COPD.

Methods: Human bronchial epithelial cells (BEAS-2B) were cultured and treated with 2% cigarette smoke medium (CSM), 1 μg/mL lipopolysaccharide (LPS) or in combination for 24 hours. Supernatant was collected and analysed by enzyme-linked immunosorbent assay for the measurements of IL-8 and MCP-1.

Results: Exposure of BEAS-2B cells to 2% CSM induced a significant increase in both IL-8 ($n = 3$; $P < 0.05$), and MCP-1 ($n = 3$; $P < 0.001$) and 1 μg/mL LPS also induced significant increase in IL-8 and MCP-1 ($n = 3$; $P < 0.001$). However, LPS-induced release of IL-8 and MCP-1 was significantly suppressed by 2% CSM ($n = 3$; $P < 0.001$).

Conclusion: Our data demonstrated that both cigarette smoke and LPS alone can induce the release of IL-8 and MCP-1. However, cigarette smoke has immunosuppressive effect as it reduces LPS-induced IL-8 and MCP-1. This immunosuppressive effect of cigarette smoke might be due to the impairment of the immune response of the airway epithelial cells, leading to bacteria colonisation in the airways, hence becoming more prone to infection.

Action of arsenic trioxide on E2F pathway and apoptosis in mesothelioma

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Introduction: Malignant pleural mesothelioma is a global health issue. Arsenic trioxide (ATO) has been shown to suppress cancer growth and induce apoptosis in acute promyelocytic leukaemia. The effect of ATO in mesothelioma was therefore studied.

Methods: A panel of five mesothelioma cell lines was used to study the effect of ATO on cell viability, pRB1, E2F1 and TYMS protein expression, and TYMS activity. Phosphatidylserine externalisation, mitochondrial membrane depolarisation, and alteration of apoptotic/anti-apoptotic proteins induced by ATO were also explored. The in-vivo effect of ATO was studied using a nude mice xenograft model.

Results: Application of ATO demonstrated anti-cancer effects in the cell line model with clinically achievable concentrations (1.7-7 μM). Downregulation of pRB1, E2F1 and TYMS protein expression as well as TYMS activity were also evident. Phosphatidylserine externalisation, mitochondrial membrane depolarisation, downregulation of Bcl-2 and Bcl-xL, and upregulation of Bak and cleaved caspase-3 were displayed. In the H226 xenograft model, the relative tumour volumes were reduced, and cleaved caspase-3 was elevated and localised to the nucleus in the ATO treatment group.

Conclusion: ATO has potent antiproliferative and cytotoxic effects in mesothelioma in vitro and in vivo by E2F1 downregulation and apoptosis. The alteration of E2F-related gene will be further investigated. There is sound scientific evidence to support the clinical application of ATO in the treatment of mesothelioma.

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Visit-to-visit blood pressure variability as a prognostic marker in patients with cardiovascular and cerebrovascular diseases: relationships and comparisons with vascular markers of atherosclerosis

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Background: Visit-to-visit blood pressure variability (BPV) is a novel risk marker for development of atherosclerotic diseases, cardiovascular and all-cause mortality. The prognostic values of BPV in comparison with other established vascular assessments remain uncertain.

Methods: We prospectively followed up 656 patients with cardiovascular or cerebrovascular diseases. Visit-to-visit BPV were recorded during a mean of 18 ± 9 outpatient clinic visits. Brachial-artery flow-mediated dilatation, carotid intima-media thickness (IMT) and plaque burden, ankle-brachial index, and arterial stiffness were determined at baseline.

Results: After a mean follow-up of 81 ± 12 months, 123 patients (19% of the study population) developed a major adverse cardiovascular event (MACE). Patients who developed a MACE had significantly higher BPV, more severe endothelial function, arterial stiffness and systemic atherosclerotic burden compared to patients who did not develop a MACE (all $P < 0.01$). BPV significantly correlated with all of the vascular assessments ($P < 0.01$). A high carotid IMT had the greatest prognostic value in predicting development of a MACE (area under receiver operating characteristic curve [AUC], 0.69 ± 0.03; $P < 0.01$). A high BPV also had moderate prognostic value in the prediction of MACE (AUC, 0.65 ± 0.03; $P < 0.01$). After adjustment of confounding factors, a high BPV remained a significant independent predictor of MACE (hazard ratio = 1.49; 95% confidence interval, 1.02-2.18; $P < 0.05$). Combination of vascular markers (such as carotid plaque and arterial stiffness) with systolic BPV provided incremental value in MACE prediction compared to either marker alone.

Conclusions: Compared with established surrogate markers of atherosclerosis, visit-to-visit BPV provides similar prognostic information and may represent a new and simple marker for adverse outcomes in patients with vascular diseases.

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Long-term prognostic implications of visit-to-visit blood pressure variability in patients with ischaemic stroke

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Background: Both blood pressure (BP) and its variability (BPV) are established risk factors for the development of atherosclerotic diseases and are associated with an increased risk of cardiovascular and all-cause mortality. The long-term prognostic implications of out-patient clinic visit-to-visit BPV among patients with ischaemic stroke are nevertheless unknown.

Methods: We prospectively followed up the clinical outcome of 632 consecutive ischaemic stroke patients without atrial fibrillation. The mean BP and BPV, as determined by the coefficient of variation of the systolic and diastolic BP, were recorded during a mean of 12 ± 6 outpatient clinic visits.

Results: The mean age of the patients was 71 ± 11 years. After a mean of 76 ± 18 month's follow-up, 161 (26%) patients died, 35% (56/161) were due to cardiovascular causes. 16% and 5% developed recurrent stroke and acute coronary syndrome (ACS), respectively. After adjusting for mean systolic BP and confounding variables, patients with a high systolic BPV were at significantly greater risk of cardiovascular mortality (hazard ratio [HR] = 2.36; 95% confidence interval [CI], 1.02-5.49; P < 0.05). A high systolic BPV also predicted all-cause mortality after adjusting for mean systolic BP (HR = 1.79; 95% CI, 1.16-2.75; P < 0.05). There was no association between systolic BPV with non-fatal recurrent stroke nor non-fatal ACS. A raised diastolic BPV did not predict recurrent non-fatal stroke, non-fatal ACS nor mortality.

Conclusions: Visit-to-visit systolic BPV predicts long-term all-cause and cardiovascular mortality in patients with ischaemic stroke without atrial fibrillation, independent of other conventional risk factors including average BP control.

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Mediterranean diet reduces blood pressure variability and subsequent stroke risk in patients with coronary artery disease

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Introduction: The Mediterranean diet has been widely advocated for the prevention of cardiovascular and cerebrovascular diseases. Meanwhile, visit-to-visit blood pressure variability (BPV) is a novel risk factor for the development of atherosclerotic diseases. However, whether diet plays a role in modulating BPV remains uncertain. We investigated whether the Mediterranean diet is associated with BPV and subsequent prognosis in patients with coronary artery disease (CAD).

Methods: A total of 274 consecutive patients with stable CAD were recruited in 2005-2006. All patients underwent a food frequency questionnaire (FFQ) to delineate their dietary intake within the past 5 years. A Mediterranean Diet Score was derived based on components within the FFQ (a higher score reflecting more components of the Mediterranean diet being taken). Patients were followed up regularly every 3-4 months and their blood pressure measured during each visit. The development of acute coronary syndrome (ACS), heart failure requiring hospitalisation, stroke and cardiovascular mortality during the follow-up period was monitored.

Results: After a mean follow-up of 77 ± 12 months, 20 (7.3% of the study population), 29 (10.6%), 13 (4.7%), and 19 (6.9%) patients developed ACS, heart failure, stroke, and cardiovascular mortality, respectively. Patients who developed a stroke had a significantly lower Mediterranean Diet Score (1.7 ± 0.5 vs 2.6 ± 1.1; P < 0.01) and significantly higher visit-to-visit BPV (systolic blood pressure standard deviation 20 ± 9 mm Hg vs 14 ± 5 mm Hg; P = 0.03) compared to those who did not develop stroke. There were no differences in Mediterranean Diet Score among patients who did and did not develop ACS, heart failure, and cardiovascular mortality. A low Mediterranean Diet Score independently predicted a raised systolic BPV (B, -0.61; 95% confidence interval [CI], -1.16 to -0.07; P = 0.03). After adjustment for confounding variables, a higher Mediterranean Diet Score reduced the subsequent risk of stroke (hazard ratio [HR] = 0.34; 95% CI, 0.15-0.75; P < 0.01) whilst a high systolic BPV significantly increased the risk of stroke (HR = 1.13; 95% CI 1.03-1.24; P = 0.01).

Conclusion: In patients with CAD, adopting a Mediterranean diet reduces BPV and subsequent stroke risk.

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Visit-to-visit systolic blood pressure variability predicts all-cause and cardiovascular mortality after lacunar infarct

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Background: Both blood pressure (BP) and its variability (BPV) are established risk factors for development of atherosclerotic disease and are associated with an increased risk for cardiovascular and all-cause mortality. The prognostic implications of out-patient clinic visit-to-visit BPV among patients with lacunar infarction are nevertheless unknown.

Methods: We prospectively followed up the clinical outcome of 281 patients with lacunar infarction. The mean BP and BPV, as determined by the standard deviation of the systolic and diastolic BP, were recorded during a mean of 13 ± 6 out-patient clinic visits.

Results: The mean age of the population was 70 ± 10 years. After a mean of 78 ± 18 month's follow-up, 65 (23%) patients died, 31% (20/65) were due to cardiovascular causes. 14% and 7% developed recurrent stroke and acute coronary syndrome, respectively. After adjusting for age, sex, mean systolic and diastolic BP, cardiovascular risk factors and co-morbidities, patients with a systolic BPV of the third tertile had significantly higher risk of all-cause (hazard ratio [HR] = 1.97; 95% confidence interval [CI], 1.02-3.80; P = 0.04) and cardiovascular mortality (HR = 7.64; 95% CI, 1.65-35.41; P < 0.01) compared to those with systolic BPV of the first tertile. Nevertheless, systolic BPV did not predict recurrent stroke or acute coronary syndrome. Diastolic BPV did not predict various adverse clinical outcomes.

Conclusions: Visit-to-visit systolic BPV predicts long-term all-cause and cardiovascular mortality after lacunar infarct, independent of conventional risk factors including average BP control.

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Stroke patients with cancer are at increased risk of recurrent stroke and cardiovascular mortality

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Background: Cancer patients are at increased risk of cardiovascular and cerebrovascular events. It is unclear whether cancer confers any additional risk for recurrent stroke or cardiovascular mortality after stroke.

Methods: This was a single-centre, observational study of 1105 consecutive Chinese ischaemic stroke patients recruited from a large stroke rehabilitation unit based in Hong Kong. We sought to determine whether patients with cancer are at higher risk of recurrent stroke and cardiovascular mortality.

Results: Among 1105 patients, 58 patients (5.2%) had cancer, of whom 74% were in remission. After a mean follow-up of 76 ± 18 months, 241 patients developed a recurrent stroke: 22 in patients with cancer (38%, annual incidence, 13.94%/year), substantially more than those without cancer (21%, 4.65%/year) [P < 0.01]. In a Cox regression model, cancer, age, and atrial fibrillation were the three independent predictors of recurrent stroke with a hazard ratio (HR) of 2.42 (95% confidence interval [CI], 1.54-3.80), 1.01 (1.00-1.03), and 1.35 (1.01-1.82), respectively. Likewise, patients with cancer had a higher cardiovascular mortality compared with those without cancer (4.30%/year vs 2.35%/year; P = 0.08). In Cox regression analysis, cancer (HR = 2.08; 95% CI, 1.08-4.02), age (HR = 1.04; 95% CI, 1.02-1.06), heart failure (HR = 3.07; 95% CI, 1.72-5.47), and significant carotid atherosclerosis (HR = 1.55; 95% CI, 1.02-2.36) were independent predictors for cardiovascular mortality.

Conclusions: Cancer patients who develop ischaemic stroke are at increased risk of recurrent stroke and cardiovascular mortality.

Myasthenic crisis in patients with generalised myasthenia gravis

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Introduction: Myasthenia gravis (MG) is an important autoimmune disease causing generalised weakness and even mortality, which is amenable to immunotherapies. Myasthenic crisis (MC) is the most serious presentation of MG typically requiring ventilator support under the care of intensive care unit. We studied factors which predict development of MC in generalised MG (gMG) patients and patients' serum cytokine levels as potential biomarkers for MG exacerbation and crisis.

Methods: Records of gMG patients being cared in Queen Mary Hospital and followed up in the neurology clinic from 1976 to 2013 were revealed. Sera / plasma taken during gMG exacerbation or crises and during follow-up from stable patients were assayed for inflammatory and anti-inflammatory cytokines levels by commercially available ELISA kit. Clinical outcome was classified according to the Myasthenia Gravis Foundation of America post-intervention status on latest follow-up.

Results: A total of 116 gMG (71.6% female) with a mean onset age of 44.8 (range, 7-83) years and mean disease duration of 7.8 (range, 1-36 years) were studied. 86.7% patients were acetylcholine receptor (AChR) autoantibodies positive, 39.3% patients had thymoma and 60.9% had thymectomy. 75% patients received immunosuppressants (corticosteroid and / or azathioprine, MMF, cyclosporin A) and 96.6% patients had satisfactory or good clinical outcome. MC occurred in 34 patients (29.3%) with a mean number of 1.9. Univariate analysis revealed that patients with MC had worse clinical severity on initial presentation (P = 0.000), increased frequencies of receiving thymectomy (76.5% vs 54.3%; P = 0.026), requiring immunosuppression (91.2% vs 68.3%; P = 0.010) than patients without MC, but are indifferent in sex, onset age, frequencies of having thymoma, positivity for Tensilon test, repetitive nerve stimulation, and AChR autoantibodies. There was no difference in long-term clinical outcome between patients with and without MC. Serum / plasma levels of interleukin-17A (IL-17A) [2.46 ± 1.19 pg/mL vs 0.063 ± 0.010; P = 0.033] and interferon-γ (IFN-γ) [8.88 ± 2.08 vs 4.35 ± 0.31; P = 0.023] were higher in patients in MG exacerbation or crisis than patients with stable disease.

Conclusion: Severe clinical weakness at disease onset may predict development of MC, and high serum / plasma levels of IL-17A and IFN-γ are biomarkers for disease exacerbation or MC in gMG.

Lipocalin 14 — a novel adipokine potentially protects mice from diet-induced diabetes

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Introduction: Obesity is one of the leading causes of worldwide chronic non-communicable diseases. There is an urgent need to develop more effective therapies to combat the global pandemic of this costly disease. Previous studies in 2011 demonstrated that a newly identified lipocalin named as LCN13 have anti-diabetic activity by enhancing insulin sensitivity of liver and adipose tissues. In mouse, a putative lipocalin LCN14 are found to share high degree of homology with LCN13 (53% identity and 67% similarity). The role of LCN14 in metabolism remains to be explored.

Methods: Male wildtype C57BL/6N mice were fed with either high-fat diet or standard chow. After 16 weeks of diet treatment, the mice were sacrificed for sampling. LCN14 gene expression was detected by quantitative polymerase chain reaction analysis and protein expression of LCN14 in tissues and serum was detected by Western blotting and by enzyme-linked immunosorbent assay analysis with in-house–developed anti-LCN14 antibody.

Results: The putative lipocalin LCN14 had been experimentally proved as an adipokine. The expression of LCN14 was found to be regulated by feeding-fasting cycles in mouse. Circulating and expression level of LCN14 was found significantly repressed in the diet-induced obese and genetically diabetic-obese (db/db) mice.

Conclusion: LCN14 potentially protects mice from diet-induced diabetes by cooperating with LCN13. Both LCN13 and LCN14 are novel candidates for developing and screening of antidiabetic drugs.

Lupus low disease activity state is associated with lower adjusted mean SLEDAI and less lupus renal and haematological involvement

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Introduction: There is currently no agreed definition of a 'safe' state of systemic lupus erythematosus (SLE) despite the availability of various validated scales on its disease activity and damage. In 2013, the Asia Pacific Lupus Collaboration presented a definition of low disease activity of this condition, the Lupus Low Disease Activity State (LLDAS), which is conceptualised as "a state which, if sustained, is associated with a low likelihood of adverse outcome" and defined as:

1. SLE Disease activity Index (SLEDAI-2k) ≤4, with no SLEDAI activity in major organ systems (renal, central nervous system, cardiopulmonary, vasculitis, haemolytic anaemia, fever) and no gastro-intestinal activity;
2. No new features of lupus disease activity compared to the previous assessment;
3. SELENA-SLEDAI physician global assessment (Physician Global Assessment, scale 0-3) ≤1;
4. Current prednisolone (or equivalent) dose ≤7.5 mg daily; and
5. Well-tolerated standard maintenance doses of immunosuppressive drugs and / or approved biologic agents.

This study was a preliminary analysis of a LLDAS cohort validation study which examines prospectively whether sustained LLDAS is associated with lower disease activity, damage, and mortality.

Methods: A cross-sectional study was carried out to determine the frequency of and the factors associated with LLDAS. Consecutive patients with SLE as defined by the revised American College of Rheumatology criteria seen at the Queen Mary Hospital during the period August 2013 to October 2013 were enrolled in the study. SLEDAI scores and their components, and lupus organ manifestations between patients who achieved LLDAS or not were compared using a Wilcoxon rank-sum test.

Results: Of the 201 patients recruited, patients who achieved LLDAS had a significantly lower mean adjusted mean SLEDAI score (2.40 ± 2.33) over the past 4 years when compared with patients who failed to achieve LLDAS (adjusted mean SLEDAI = 3.86 ± 2.17; P<0.000005). Similarly, patients who achieved LLDAS had lower anti-dsDNA and higher complement levels (P = 0.0012), and lower renal (P = 0.0028) and haematological (P = 0.011) scores.

Conclusion: Preliminary analysis shows that LLDAS can discriminate patients with a relatively safe course of disease, as reflected by the association of LLDAS with lower adjusted mean SLEDAI for the past 4 years and less renal and haematological involvement. Further follow-up prospective studies may reveal whether a sustained LLDAS is associated with a better prognosis with less cumulative disease activity, damage, and lower mortality rate.

Intermittent hypoxia-induced endothelial activation in EA.hy926 cells in vitro

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Introduction: Obstructive sleep apnoea is characterised by repetitive episodes of complete or partial upper airway collapse during sleep, leading to recurrent drop in arterial oxygenation (intermittent hypoxia [IH]) and sleep fragmentation. Repetitive IH with rapid reversal to normoxia initiates a series of pathological events such as oxidative stress, inflammation, and sympathetic activation, all of which contributes to endothelial dysfunction (a predictor and precursor of atherosclerosis). The aim of the current study was to investigate the effect of IH on the activation of endothelial cells in a cellular model in vitro.

Methods: Cultured endothelial EA.hy926 cells were exposed to IH in the hypoxic chamber in which the O_2 levels were alternated between 1% for 10 minutes and 21% for 5 minutes. Cells in the control group were maintained in normoxic conditions (IN: 21% and 5% CO_2). Cells were exposed to IN or IH for 64 cycles. Expression of protein of interests in endothelial cells was analysed by Western blotting.

Results: Endothelial nitric oxide synthase (eNOS), a key enzyme regulating endothelial function, was downregulated after exposure to IH while inflammatory response proteins such as cyclooxygenase–2 and inducible NOS (iNOS) were upregulated. Increased phosphorylation of Akt [p-Akt (ser473)], Erk (p-Erk1/2), and p38 (p-p38) was observed in IH-treated cells compared to the control cells.

Conclusion: IH induces endothelial dysfunction with reduced eNOS protein expression. The expression of inflammatory proteins and activation of the specific signalling pathways suggest pathophysiological changes in response to IH in this in-vitro cellular model.

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Modelling of Friedreich ataxia–related iron overloading cardiomyopathy using patient-specific induced pluripotent stem cells

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Background: Friedreich ataxia (FRDA), a recessive neurodegenerative disorder commonly associated with hypertrophic cardiomyopathy, is due to GAA repeats expansion within the first intron of the frataxin (*FXN*) gene encoding the mitochondrial protein involved in iron-sulfur cluster biosynthesis. The triplet codon repeats leads to heterochromatin-mediated gene silencing and loss of frataxin. Nevertheless, inadequacy of existing FRDA-cardiac cellular models limited the cardiomyopathy studies. We tested the hypothesis that iron homeostasis deregulation accelerates reduction in energy synthesis dynamics which contributes to impaired cardiac calcium homeostasis and contractile force.

Methods and Results: Silencing of *FXN* expressions occurred both in somatic FRDA-skin fibroblasts and two of the iPSC clones. A sign of stress condition was shown in FRDA-iPSC-cardiomyocytes with disorganised mitochondrial network and mitochondrial DNA depletion. Hypertrophic cardiac stress responses were observed by increased alpha-actinin-positive cell sizes revealed by FACS analysis as well as elevation in brain natriuretic peptide (BNP) gene expression. The intracellular iron accumulated in FRDA-cardiomyocytes might be due to attenuated negative feedback response of transferring receptor (TSFR) expression and positive feedback response of ferritin (FTH1). Energy synthesis dynamics, in terms of ATP production rate, was impaired in FRDA-iPSC cardiomyocytes, which were prone to iron overload condition. Energetic insufficiency determined slower Ca^{2+} transients by retarding calcium reuptake to sarcoplasmic reticulum and impaired the positive inotropic and chronotropic responses to adrenergic stimulation.

Conclusion: Our data showed for the first time that FRDA iPSCs cardiac derivatives represent promising models to study cardiac stress response due to impaired iron-homeostasis condition and mitochondrial damages. The cardiomyopathy phenotype was accelerated in iron-overloaded condition early in calcium homeostasis aspect.

A study of the rehabilitation outcomes of stroke survivors after thrombolytic therapy

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Introduction: Thrombolytic therapy is now a standard therapy for acute ischaemic stroke patients who met eligible criteria. The rehabilitation outcomes of stroke survivors after thrombolytic therapy were scarcely reported in Hong Kong. This study aimed to fill up this gap in knowledge.

Methods: This was a retrospective descriptive study. A total of 29 ischaemic stroke patients who had received thrombolytic therapy and been transferred to the Medical Rehabilitation Unit of Tung Wah Hospital for in-patient rehabilitation between July 2009 and December 2012 were recruited.

Results: Thirteen female and 16 male patients were enlisted for study with a mean age of 71 (range, 52-91) years. There were 13 (44.8%) cortical, 11(37.9%) subcortical, and 5 (17.3%) brainstem strokes. The mean length-of-stay (LOS) as a whole was 30.1 days which did not significantly differ from the historical control in 2011. Detailed subgroup analysis revealed that cortical stroke patients performed less well when compared with subcortical and brainstem stroke patients. LOS for cortical, subcortical, and brainstem strokes were 39, 27.5, and 13 days, respectively. All three subgroups showed significant gains in Functional Independence Measure (FIM) scores after rehabilitation. Cortical stroke patients have a statistically significant lower admission FIM score (47.2) and discharge FIM score (62.9) than subcortical (admission / discharge, FIM: 72.6 / 90.7) and brainstem stroke patients (admission / discharge, FIM: 68.2 / 79.2). Cortical stroke patients have a lower rate of home discharge than non-cortical stroke patients (54% vs 81%, $P < 0.05$). Home discharge patients were associated with higher discharge FIM scores than those discharged to institution (84.9 vs 59.8, $P < 0.01$).

Conclusion: Stroke survivors with thrombolytic therapy did improve significantly after rehabilitation in terms of FIM measures. Favourable rehabilitation outcomes (shorter LOS and home discharge) were associated with a non-cortical stroke nature and a higher discharge FIM scores. Further case-control and prospective studies can delineate better the contribution of thrombolytic therapy on rehabilitation outcomes.

Role of bone morphogenetic protein-7 in diabetic nephropathy

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Introduction: Bone morphogenetic protein-7 (BMP7) has been reported to confer renal protected effects in a variety of cell types and disease model, but the potential role of BMP7 in diabetic nephropathy remains unknown.

Methods: Nine-week-old *db/db* mice and their *db/m* littermates underwent uninephrectomy (Unx) or sham operation, and received rh-BMP7 (300 μg/kg body weight) or vehicle intraperitoneally every other day for 8 weeks before sacrifice. 24-Hour urine and blood samples of mice were collected every 4-week interval.

Results: Compared with vehicle control, Unx *db/db* mice treated with rh-BMP7 had significantly lower urinary albumin-to-creatinine ratio (4566 ± 2767 μg/mg vs 7338 ± 5748 μg/mg; $P < 0.05$), serum blood urea nitrogen (33.3 ± 3.46 mg/dL vs 37.5 ± 2.95 mg/dL; $P < 0.05$), and renal cortical expression of ICAM-1 and CCL-2 in both gene and protein levels. PAS staining of kidney tissue showed significantly less severe glomerular and tubular damage and interstitial inflammatory cell infiltration in the BMP7-treated group. Western Blotting of kidney cortex showed significant increase of p38 and p44/42 MAPK phosphorylation in Unx *db/db* vehicle group while treated with BMP7 suppressed their phosphorylation.

Conclusions: Our results demonstrated for the first time that BMP7 reserved renal function and attenuated pro-inflammatory responses in diabetic kidney by suppressing multiple signalling pathways including p38 and p44/42 MAPK. Its potential application as a therapeutic molecule in diabetic nephropathy warrants further investigation.

Acknowledgement: This study was supported by a General Research Fund of the Research Grants Council (Grant number: HKU7770/09M) of Hong Kong.

Differential effects of epigallocatechin gallate on cigarette smoke-induced upregulation of CINC-1 and IL-6 in Rat H9c2 cardiomyocytes

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Introduction: Cigarette smoke (CS) containing numerous harmful substances is considered to precipitate spasm of micro vessels, which is supported as a cause of idiopathic-dilated cardiomyopathy, as well as have a toxic effect on the myocardium. The mechanism is currently unclear, but both oxidative stress and inflammatory responses may play an essential role in the CS-induced biological processes. Several antioxidant agents have been used in the control of the inflammatory responses. The aim of this study was to investigate the effect of epigallocatechin gallate (EGCG), the major component of polyphenols in green tea, on CS-induced inflammatory responses in rat H9c2 cardiomyocyte model.

Methods: The H9c2 cell line was cultured in DMEM containing 10% fetal bovine serum, in a CO_2 incubator at 37°C. When cells reached 80% confluence, the medium was replaced with experimental medium consisting of 1% fetal bovine serum 24 hours before treatment. Cigarette smoke medium (CSM) was prepared by bubbling smoke from two cigarettes into 20 mL serum-free medium, which was regarded as 100%. Cells were pretreated with 0.1, 1, or 10 μM EGCG for 30 minutes before 4% CSM was added and incubated for an additional 24 hours. Supernatant was collected for determination of interleukin-6 (IL-6) and cytokine-induced neutrophil chemoattractant-1 (CINC-1) by ELISA.

Results: CSM caused elevation of pro-inflammatory markers IL-6 and CINC-1 (resemble to human IL-8) in H9c2 cells. EGCG alone also caused IL-6 elevation in a dose-dependent manner. There was a significant inhibitory effect of EGCG at low dose (0.1 μM) observed on CSM-induced elevation of IL-6. However, EGCG alone or in combination of CSM had no effects on CINC-1 level.

Conclusion: Our findings suggest that low dose of EGCG treatment may alleviate the production of pro-inflammatory cytokine IL-6 but not CINC-1, indicating that the release of pro-inflammatory cytokines is under different transcriptional control in rat H9c2 cardiomyocytes.

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New high-definition narrow band imaging versus conventional high-definition white light colonoscopy for detection of colorectal adenomas: a randomised controlled trial with tandem colonoscopy

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Introduction: Adenoma detection is important in colonoscopy as polypectomy has been shown to reduce the subsequent incidence and mortality of colorectal cancer. Narrow band imaging (NBI), an image-enhanced imaging system of the endoscopy, is developed to improve the diagnostic performance of the endoscopy. We tested whether the new generation of NBI colonoscopy would improve detection of colorectal adenoma when compared with high-definition white light (HD-WL) in a randomised tandem colonoscopy study.

Methods: Patients were recruited from those undergoing scheduled colonoscopy for symptoms, screening, or surveillance. Colonoscopists involved were all experienced operators. Patients were randomised to the new NBI or HD-WL colonoscopy. Tandem colonoscopy was immediately performed in all patients by using the same assigned colonoscope. NBI was used on both withdrawals in the new NBI group and standard WL examination was used in the HD-WL group. The primary endpoint was adenoma detection rate, which was defined as the proportion of patients with at least one adenoma detected on first pass examination. Lesions detected on second-pass examination were considered to be missed lesions.

Results: A total of 360 patients were randomised to receive the new NBI or HD-WL. Both the adenoma and polyp detection rates were significantly higher in the NBI group than in the HD-WL group (adenoma: 48.3% vs 34.4%, P = 0.01; polyps: 61.1% vs 48.3%, P = 0.02). The mean number of polyps detected per patient tended to be higher in the NBI group (1.49 vs 1.13, P = 0.07). There was no significant difference in the adenoma miss rates between the two groups (21.8% vs 21.2%). Eleven (6.5%) patients in the new NBI group and 16 (9.7%) patients in the HD-WL group were found to be adenoma on tandem colonoscopy only (P = 0.32). Multivariate analysis found that the use of NBI (odds ratio [OR] = 2.09; 95% CI, 1.32-3.30), increasing age (OR = 1.05; 95% CI, 1.03-1.07), and male patients (OR = 3.03; 95% CI, 1.92-4.78) were associated with adenoma detection.

Conclusion: Our results suggested that the new NBI was superior to the conventional HD-WL in detecting colorectal adenoma.

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Effects of cigarette smoke on 5-hydrotrytamine metabolism in human bronchial epithelial cells

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Introduction: Serotonin (5-hydrotrytamine, 5-HT) plays an important role in pulmonary functions. It is synthesised from tryptophan by aromatic 1-amino acid decarboxylase (AADC) and released at the sites, which binds to 5-HT receptors at the postsynaptic ends. Excess or turnover 5-HT will be recycled back into cells via 5-HT reuptake transporter (SERT), stored or subsequently metabolised by monoamine oxidase A (MAO-A) inside the cells. With the influence of smoking on serotoninergic neurons, we hypothesised that there may be a potential 5-HT analogue in cigarette smoke. We aimed to study the effect of 5-HT on interleukin (IL)–8 release and the effects of cigarette smoke on the metabolism of 5-HT in human bronchial epithelial cells.

Methods: Serotonin in aqueous phase cigarette smoke solution was firstly determined by HPLC (Waters) with fluorometric detection and further confirmed by The ESI–MS and MS/MS chromatography. Cigarette smoke medium (CSM) was generated by bubbling smoke from two cigarettes into 20 mL serum-free medium, which was regarded as 100%. The human bronchial epithelial cell line (BEAS-2B) was cultured to 80% confluence in complete keratinocyte-SFM before treatment with either 5-HT or 2% CSM. Release of a pro-inflammatory marker, IL-8 was determined by ELISA. MAO-A and MAO-B activity were measured by MAO Glo assay kit (Promega, WA, US).

Results: A 5-HT analogue in aqueous phase cigarette smoke was discovered using LC-MS/MS approach. In support, exposure to 5-HT caused elevation of IL-8 at both mRNA and protein levels. Reduced monamine oxidase activity was observed after treatment with CSM, indicating the presence of 5-HT.

Conclusion: Our data suggest that accumulation of 5-HT in bronchial epithelia may occur in smokers, resulting in chronic inflammation in patients with chronic obstructive pulmonary disease.

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A needs assessment and review curriculum of content of teaching of teaching on systemic lupus erythematosus

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Background: Systemic lupus erythematosus (SLE) is an autoimmune disease that is more common among Asians compared with Whites but the prevalence is significantly low. This study aimed to evaluate medical students' knowledge on SLE and their feedback on teaching on SLE-related topics at the University of Hong Kong.

Methods: Senior medical students were recruited to complete a self-administered questionnaire regarding learning, teaching, and subjects regarded as important in SLE.

Results: A total of 124 (109 MBBS IV, 15 MBBS V) medical students were recruited. A majority of students acquired knowledge on SLE from standard sources provided by curriculum teaching including lectures (98.4%), textbook (96.7%), patient contact (86.1%), and problem-based learning sessions (64.8%). A significant proportion of students (77.0%) also obtained knowledge from internet and e-learning. An addition of 36.4% of students also preferred learning from extra-curricular sources including education leaflets from professional societies, family contact, and television programme. Most students regarded knowledge on clinical presentations (45.4%), diagnosis (49.6%), management and treatment (76.5%) as the most fundamental knowledge an average doctor should know more about SLE. Renal disease was considered most important (36.7%) among all organ involvement in SLE teaching. Only 8.4% of students regarded epidemiology and pathogenesis as essential but overall importance of subjects on epidemiology and pathogenesis, management and complications ranked 4.0/5, 4.3/5 and 4.2/5, respectively. These senior medical students graded their confidence in knowledge in SLE as 3.4/5. In general, the quality (3.9/5) and quantity (3.5/5) of teaching regarding SLE is good.

Conclusion: A majority of students were satisfied with current curriculum teaching and valued clinical management with higher priority than epidemiology and pathogenesis among the taught subjects. Extra-curricular sources of learning including information from professional societies and rheumatology nurse may be considered an adjunct to teaching.

Generation of LAMP2-deficient patient-specific induced pluripotent stem cells for modelling Danon disease

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Introduction: Danon disease (DD) is a dominant X-linked disorder caused by the loss-of-function mutation of the *LAMP2* gene. The LAMP2 protein plays an important role in mediating autophagy. Deficiency in LAMP2 thus leads to the accumulation of obsolete materials in the affected cells. Clinically, affected individual will develop severe cardiomyopathy. Unfortunately, there is no effective therapy for DD due to the lack of a human cardiomyocyte-based model to reveal the underlying pathogenic mechanism. Addressing this issue, we have collected the skin fibroblasts from patients carrying a non-sense LAMP2 mutation and reprogrammed them into induced pluripotent stem cells (iPSCs). The resultant patient-specific iPSCs could be differentiated into functional cardiomyocytes for disease modelling purpose.

Methods: Skin fibroblasts from DD patients ($LAMP2_{Q174X}$ mutant) were reprogrammed into iPSCs with the standard Yamanaka procedures. After characterisation, the DD-iPSCs were differentiated into cardiomyocytes for structural (immunostaining and electron microscopy) and functional analysis (calcium handling and autophagy status).

Results: Compared to the normal iPSCs-derived cardiomyocytes, excessive accumulation of glycogens and autophagic materials were observed in DD-iPSCs–derived cardiomyocytes. Functionally, the DD-iPSCs–derived cardiomyocytes showed impaired calcium handling that contributed to the observed isoproterenol induced cardiac arrest.

Conclusion: The LAMP2-deficient iPSCs has been generated from the skin fibroblasts isolated from DD patients. These iPSCs were differentiated into functional cardiomyocytes for disease modelling and drug testing.

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Cyclin-dependent kinases regulatory subunit 2 (CKS2) counteracts CKS1 to regulate degradation of p27

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Introduction: Cyclin-dependent kinase subunit (CKS) proteins are evolutionarily conserved cyclin-dependent kinase (CDK) subunits whose functions are incompletely understood. Mammals have two CKS proteins. CKS1 acts as a cofactor to the ubiquitin ligase complex SCF(SKP2) to promote degradation of CDK inhibitors, such as p27. Little is known about the role of the closely related CKS2.

Methods: Cell cycles of CKS1 knock-out (-/-) mouse embryonic fibroblasts (MEFs) and CKS2-/- MEFs were checked. p27 levels in both MEFs would also be checked by western blot. By immunoprecipitation, p27 ubiquitylation levels in wild-type (WT), CKS1(-/-) and CKS2(-/-) would be compared. Cyclin A/CDK2 activities in MEFs would be checked as well.

Results: CKS1-/- MEFs showed a largely widened G1 and S phase while CKS2-/- MEFs showed a reduced G1 population and an increased fraction of cells in S phase. And the p27 level in CKS1-/- is much higher than WT MEFs and CKS2 MEFs showed the opposite. Such data were also supported by the ubiquitin level of p27 in both MEFs (higher ubiquitin level in CKS2-/- and lower level in CKS1-/- when compared with WT MEFs). Cyclin A/CDK2 activity in CKS2-/- MEFs was also much higher.

Conclusion: We conclude that CKS2 counteracts CKS1 and stabilises p27. Unopposed CKS1 activity in Cks2(-/-) cells leads to loss of p27. The resulting unrestricted cyclin A/CDK2 activity is accompanied by shortening of the cell cycle.

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Interim analysis of hepatitis B reactivation in patients with prior hepatitis B virus exposure undergoing rituximab-containing chemotherapy: a prospective study

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Background: Patterns of hepatitis B virus (HBV) reactivation in hepatitis B surface antigen (HBsAg)–negative antibody to the hepatitis B core antigen (anti-HBc)–positive individuals receiving rituximab-containing chemotherapy have not been well described.

Methods: From October 2011 onwards, we recruited HBsAg-negative, anti-HBc–positive Chinese patients with baseline undetectable serum HBV DNA (<10 IU/mL), diagnosed with haematological malignancies and receiving rituximab-containing chemotherapy. Subjects were prospectively monitored every 4 weeks after the first dose of rituximab up to 2 years from recruitment. Entecavir was started when detectable HBV DNA (≥10 IU/mL) was encountered.

Results: Up till September 2013, among 252 patients receiving rituximab-containing chemotherapy, 67 (30.3%) were HBsAg-negative, anti-HBc–positive, of which 62 (38.7% male) patients with undetectable viremia at baseline were recruited. 48 (77.4%) had baseline detectable anti-HBs (range, 11-683 mIU/mL). The 1-year cumulative rate of HBV reactivation, calculated using the Kaplan-Meier method, was 29.3%. The median HBV DNA level at reactivation was 28.2 (range, 16-920) IU/mL. Reactivation was significantly more common within the first 6 months of rituximab commencement when compared to the period of 6 months to 1 year (P = 0.045). Baseline anti-HBs–negative subjects, compared to anti-HBs–positive subjects, had a significantly higher cumulative reactivation rate (57.8% and 17.5% respectively, P = 0.019). Lower levels of baseline anti-HBs were associated with a higher rate of reactivation (P = 0.015).

Conclusion: High rates of HBV reactivation were observed in HBsAg-negative, anti-HBc–positive individuals undergoing rituximab-containing chemotherapy within the first year of therapy commencement, with the majority occurring within the first 6 months. Low baseline anti-HBs levels were associated with an increased rate of reactivation. Entecavir treatment controlled HBV reactivation in all cases. (ClinicalTrials.gov identifier NCT01502397)

57 Cerebrospinal fluid biomarkers of Alzheimer's disease in Chinese patients: a pilot study

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Introduction: In view of the paucity of data on cerebrospinal fluid (CSF) biomarkers in Chinese patients, we evaluated the validity of tau, phosphorylated tau 181 (p-tau), amyloid beta 42 (Aβ42), and Aβ40 proteins in Chinese Alzheimer's disease (AD) patients.

Methods: We recruited 24 AD patients, 12 non-demented controls, and 12 non-AD dementia subjects from memory clinic or medical general ward of Queen Mary Hospital.

Results: We found the CSF levels of Aβ42, tau, p-tau, Aβ42/tau and Aβ42/p-tau ratios, except the Aβ40 protein level, were significantly different among the three groups of subjects. AD subjects had higher levels of CSF tau and p-tau, but lower levels of Aβ42 proteins, Aβ42/tau and Aβ42/p-tau ratios, than non-demented controls. For the diagnosis of AD versus non-demented controls, the sensitivity and specificity of the ratios of Aβ42/tau and Aβ42/p-tau were 96%, 83%, 92% and 83%, respectively. Only the Aβ42/p-tau ratio showed satisfactory sensitivity and specificity for the diagnosis of AD versus other dementia subjects.

Conclusions: Chinese AD patients have low CSF Aβ42 levels and elevated CSF tau and p-tau levels. A combination of these three CSF biomarkers is useful in the diagnosis and differential diagnosis of AD in Chinese.

58 The prevalence of cognitive impairments by Mini-Mental State Examination in Chinese patients with end-stage renal failure on peritoneal dialysis

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Objective: The prevalence of cognitive impairment among patients with end-stage renal failure (ESRF) ranges from 16 to 38%, which is 3 times higher than age-matched general population in western countries. We investigated the local prevalence of cognitive impairment in incident peritoneal dialysis (PD) Chinese ESRF patients and explored its relationship to PD-related peritonitis in the first 6 months of PD treatment.

Methods: A total of 92 patients were newly started on PD between February 2011 and March 2013. The Mini-Mental State Examination was performed to assess any cognitive impairment, using education-adjusted cut-offs. Data on patient characteristics including demographics, co-morbidities, blood biochemistry, dialysis adequacy, medications, and PD peritonitis were collected.

Results: The mean age was 59 ± 14.8 years; 47.8% were females. The frequencies of diabetes mellitus, hypertension, ischaemic heart disease, peripheral vascular disease, and history of stroke were 38%, 93.5%, 23.9%, 10.9% and 8.7%, respectively. Residual renal function (glomerular filtration rate) was 3.6 ± 2.7 mL/min and Kt/V being 2.12 ± 0.465. The prevalence of cognitive impairment was 12%. Old age and female sex were independent risk factors for cognitive impairments ($P = 0.031$ and $P = 0.036$, respectively) in logistic regression analysis. 20.7% of patients developed peritonitis (1 episode per 23 patient-months) within the first 6 months of PD. Patients with cognitive impairment had a higher rate of PD peritonitis than those without cognitive impairment (1 episode per 11 vs 1 episode per 27 patient-months respectively, $P = 0.033$). Old age was the only independent risk factor for peritonitis, while cognitive impairment showed a non-significant trend towards an increased risk ($P = 0.031$ and $P = 0.072$, respectively).

Conclusion: Cognitive impairment is common in incident PD patients locally and may be a risk factor for PD peritonitis.

The role of inka1b in zebrafish haematopoiesis

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Introduction: *Inka1b* gene was shown up-regulated in the intermediate cell mass (ICM) of the zebrafish chordin morphants but its role in embryonic haematopoiesis is unknown. In this study, we characterised the spatio-temporal expression pattern and haematopoietic functions of inka1b in zebrafish embryos.

Methods: Spatio-temporal expression of zebrafish inka1b gene was examined by reverse transcription polymerase chain reaction (RT-PCR) and whole-mount in-situ hybridisation (WISH). *Inka1b* was knocked down by anti-sense morpholino (MO) [herein inka1b^{MO} embryos] and the haematopoietic phenotype was analysed by WISH, real-time RT-PCR, O-dianisidine, Sudan Black staining and quantitatively by flow cytometry in *Tg(mpo:EGFP)* embryos.

Results: INKA is present in vertebrates and relatively conserved from human to zebrafish. In zebrafish embryos, inka1b was expressed since the zygotic stage, indicating the presence of maternal transcript. At 18 and 24 hpf, *inka1b* could be detected in the ICM in wild-type and chordin morhants. Erythropoiesis was reduced in inka1b^{MO} embryos, as shown by reduction of *gata1* and alpha-embryonic haemoglobin expression as well as O-dianisidine staining at 48 hpf and the haematopoietic defects could be rescued by co-injection with wild-type *inka1b* mRNA. Differentiation was not affected and cytological examination of erythrocytes in inka1b^{MO} embryos showed no difference from those in un-injected control. *Inka1b* knock-down also resulted in modest up-regulated expression of myelomonocytic markers *pu.1* and *l-plastin* but reduced granulopoiesis as confirmed by RT-PCR, WISH and flow cytometric analysis in *Tg(mpo:EGFP)* embryos. Definitive haematopoiesis and angiogenesis were not affected.

Conclusion: Zebrafish *inka1b* is involved in the maintenance of erythropoiesis and macrophage in primitive haematopoiesis.

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Adipocyte fatty acid binding protein is a potential regulator of adaptive thermogenesis

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Introduction: Adipocyte fatty acid binding protein (A-FABP) is a well-known pro-inflammatory adipokine that links obesity with its related diseases. Previous studies show that A-FABP knockout (KO) mice are more susceptible to diet-induced obesity comparing to wild type (WT) littermates. As defective adaptive thermogenesis is one of the major contributors to diet-induced obesity, here we investigate the role of A-FABP in the regulation of adaptive thermogenesis.

Methods: A-FABP KO mice and their WT littermates were fed on either standard chow (STC) or high-fat high-cholesterol (HFHC) diet for 27 weeks. Cold challenge study was performed to evaluate the ability of thermoregulation of mice. Western blot analysis was performed to determine the expression of various proteins (UCP-1, AMPK, HSL, ATGL) in brown adipose tissue (BAT).

Results: A-FABP expression in BAT and its circulating level were significantly elevated in WT mice in response to HFHC diet induction and acute cold exposure. HFHC diet–induced A-FABP KO mice were cold intolerant while STC-fed A-FABP KO mice showed better thermoregulation comparing to their relative WT controls. The basal UCP-1 expression in BAT was significantly higher in A-FABP KO mice and was accompanied with higher basal expression of AMPK, HSL, ATGL when compared to WT mice. However, both HFHC diet–induced and cold-induced up-regulation of UCP-1 expression were impaired in A-FABP KO mice comparing to WT mice.

Conclusion: These data suggest that A-FABP is involved in both cold- and diet-induced adaptive thermogenesis and the up-regulation of basal UCP-1 expression in A-FABP KO mice may be a compensatory mechanism for maintaining energy homeostatsis.

A novel C-type lectin receptor, CLEC16A, and systemic lupus erythematosus

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Introduction: C-type lectin receptors (CLRs) are pattern recognition receptors that sense microbial pathogens and other forms of danger, leading to the induction of inflammatory responses in the host that link innate to adaptive immunity. Abnormalities in expression of CLRs can lead to various autoimmune diseases, such as systemic lupus erythematosus (SLE). Polymorphisms within the C-type lectin domain family 16 member A (*CLEC16A*) gene, especially those situated in introns 19 and 22, have been found to be significantly associated with SLE in both Chinese and Caucasians. However, functional studies of CLEC16A protein have been lacking; only two have reported on the role of the Drosophila ortholog of mammalian CLEC16A in autophagosomal pathway. The current project was undertaken to dissect the physiological roles of CLEC16A in mammalian cells.

Methods: The project was divided into two parts. First, peripheral blood mononuclear cells (PBMC) were collected from normal and SLE individuals to compare the expression levels of the two naturally occurring isoforms of *CLEC16A* in healthy and disease states by real-time polymerase chain reaction. Second, the cDNA of CLEC16A was cloned and overexpressed in HeLa cells. Overexpressing HeLa cells were subjected to immunofluorescence (IF) microscopy to identify the compartments where CLEC16A might be located.

Results: The expression levels of both isoforms of *CLEC16A* in PBMC were found to be lower in SLE patients compared to healthy controls. In particular, the expression of isoform 1 was found to negatively correlate with cumulative SLE disease activity index (SLEDAI). In overexpression studies, CLEC16A showed a punctated expression pattern in HeLa cells under resting state. CLEC16A did not reside in the lysosomal pathway as it did not co-localise with either EEA-1 (early endosomal marker) or LAMP-1 (late endosomal marker).

Conclusions: The expression of *CLEC16A* in PBMC appeared to correlate with SLE disease status, particularly isoform 1, which was expressed at lower levels as SLEDAI went up. The punctated expression pattern of CLEC16A suggested that it was located within an organelle under resting state. Further IF co-localisation experiments with various organelle markers would be conducted to identify the specific cellular compartment(s) where CLEC16A resided. Such knowledge would be instrumental clues for dissecting the roles that CLEC16A holds in the mammalian cells.

Gastro-oesophageal reflux disease—a decade later: rising population prevalence in a Chinese population

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Objective: Gastro-oesophageal reflux disease (GERD) prevalence is consistently lower in Chinese than in Caucasian populations. Population-based data tracking the prevalence of GERD over the last decade in Chinese subjects suggest that the prevalence of GERD is rising. The prevalence and risk factors associated with an epidemiological diagnosis of GERD were examined. Prevalence rates in 2002, 2006, and 2011 were compared.

Methods: A total of 3360 Chinese subjects were polled in a telephone survey using a validated GERD questionnaire and the Hospital Anxiety and Depression Scale. Prevalence rates in 2011 were compared with prevalence rates in 2002 and 2006. A sensitivity analysis was undertaken to determine whether utilisation of the Montreal definition of weekly GERD versus symptomatic weekly GERD would alter the interpretation of weekly GERD prevalence rates in 2011. Multiple logistic regression was performed to determine the risk factors associated with weekly GERD.

Results: A total of 2074 subjects (mean age, 48.1 ± 18.2 years; range, 18-94 years; 63.1% female) completed the survey (response rate, 61.7%). The prevalence of GERD as defined by the Montreal definition was 3.8%. The prevalence of moderate-to-severe GERD at least daily, weekly, monthly, and yearly was 0.9%, 2.8%, 6.6%, and 12.5%, respectively. The prevalence of weekly GERD had increased by 1.3% between 2002 and 2011, which represents an at least 50% relative increase. A diagnosis of weekly GERD was associated with non-cardiac chest pain (odds ratio [OR] = 1.7; 95% confidence interval [CI], 1.034-2.9; P = 0.037), dyspepsia (OR = 5.1; 95% CI, 3.0-8.8; P < 0.005), an acid feeling in the stomach (OR = 3.0; 95% CI, 1.8-5.1), and a need to raise the pillow to sleep (OR = 1.8; 95% CI, 1.017-3.1; P = 0.044). Variables associated with a diagnosis of GERD are similar to a decade ago.

Conclusion: GERD rates in the ethnic Chinese are consistently lower than those in western populations; however these rates have risen over the last decade. Despite this, variables associated with a survey diagnosis of GERD remain ostensibly unchanged.

Excess fructose, lactose, oligosaccharides, and polyols in the Chinese diet: a prospective qualitative study on dietary FODMAP content

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Background: The emergence of FODMAP restriction in the management of irritable bowel syndrome (IBS) has been a seminal development. Little is known about whether the Chinese diet contains high FODMAPs foods that could be restricted to effect symptoms management in functional gastrointestinal disorders (FGID). This study aimed to examine the possible sources of FODMAPs in the Chinese diet and to identify food items that warrant FODMAP analysis in this region.

Methods: Consecutive subjects from the gastro-intestinal out-patients of a major teaching hospital in Hong Kong were recruited if they had a diagnosis of functional dyspepsia, IBS, or inflammatory bowel disease. Control subjects were recruited from volunteers or relatives of patients at the hospital. Subjects were asked to keep a prospective diary of their entire food and fluid intake over a consecutive 5-day period. Subjects were asked to measure serving portions and to record the components of their meals to facilitate nutritional and FODMAP analysis.

Results: The diets of a total of 25 subjects were studied: 7, 6, 6, and 6 with functional dyspepsia, irritable bowel disease, inflammatory bowel disease, and control subjects, respectively. The mean age was 50.4 years and mean body mass index was 22.5 kg/m^2. The mean calorie intake was 1666.8 calories (95% confidence interval [CI], 1543.9-1789.7). The relative proportion of fat, carbohydrate, and protein intake were 14%, 61% and 25%, respectively. The mean number of items with high FODMAP content ingested on a daily basis was 5.4 (95% CI, 4.8-6.1). The two main elements of the FODMAP complex ingested were oligosaccharides (found in wheat products, onions, garlic, and bean) and disaccharides (found in milk, milk powder, and butter) representing 62.22% (95% CI, 57.8-66.6%) and 26.6% (95% CI, 22.6-30.6%) of the relative proportion of FODMAP items ingested, respectively. This study also found dinner was the only meal where rice was clearly the main cereal ingested, at other meals wheat ingestion predominated with the exception of lunch where wheat and rice product ingestion were almost equal.

Conclusions: This study is the first to suggest that there are significant sources of FODMAPs in the Chinese diet. The study also confirms the significant westernisation of the Chinese diet in Hong Kong. Subjects with FGID such as IBS may benefit from selective restrictions of these carbohydrates.

Antiplatelet drug resumption after antiplatelet-related intracerebral haemorrhage

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Introduction: Antiplatelet (AP) drugs resumption in survivors of AP-related intracerebral haemorrhage (AICH) represents an important medical dilemma as these patients have a high risk for both recurrent intracerebral haemorrhage (ICH) and ischaemic vascular event. The increased risk and high mortality of recurrent ICH is a significant factor that leads to the reluctance among clinicians to resume AP in survivors of AICH.

Methods: Medical records of consecutive survivors of AICH with standard indication for AP admitted from 1 July 2002 till 30 June 2010 were reviewed. The primary end-point was vascular death (death due to recurrent ICH or ischaemic vascular event). Other end-points were recurrent ICH and ischaemic vascular event. Univariate hazard ratio for vascular death and recurrent ICH were derived from a Cox proportional hazards model.

Results: There were 96 survivors. The mean age was 72.9 years and 66.7% of the survivors were male. A total of 35 patients (36.5%) were subsequently prescribed AP (aspirin = 33, clopidogrel = 2), in which 13 were prescribed after an ischaemic vascular event. Among AP users, there were 3 vascular deaths (rate, 29.8 per 1000 patient-years; 95% confidence interval [CI], 6.1-87.0), 4 recurrent ICH (rate, 39.7 per 1000 patient-years; 95% CI, 10.8-101.6), and 5 ischaemic vascular events (rate, 61.1 per 1000 patient-years; 95% CI, 19.8-142.5). Among non-AP users, there were 7 vascular deaths (rate, 26.1 per 1000 patient-years; 95% CI, 10.5-53.7), 4 recurrent ICH (rate, 15.4 per 1000 patient-years; 95% CI, 4.2-39.4), and 25 ischaemic vascular events (rate, 101.9 per 1000 patient-years; 95% CI, 66.0-150.5). AP exposure was not associated with vascular death (hazard ratio = 1.12; 95% CI, 0.29-4.36; P = 0.869). Hazard ratios for recurrent ICH were 2.24 (95% CI, 0.56-8.88; P = 0.255) for AP exposure, and 0.32 (95% CI, 0.08-1.26; P = 0.105) for index AICH at a deep hemispheric location.

Conclusion: AP resumption after AICH was not associated with an increased risk of vascular death. In view of the high rate of ischaemic vascular event among survivors of AICH, AP resumption should be considered, especially in survivors with lower risk of recurrent ICH.

Cholesterol transport proteins and diabetic nephropathy

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Introduction: Lipid accumulation in the kidney has been shown to accelerate renal injury. Intracellular accumulation of lipids may be caused by alterations in synthesis as well as lipid uptake and efflux. Cellular cholesterol efflux is mediated by specific cholesterol transport proteins including adenosine triphosphate–binding cassette transporter A1 (ABCA1), ABCG1, and scavenger receptor class B type I (SR-BI). We have investigated whether there are changes in cellular cholesterol transport proteins in diabetic nephropathy (DN).

Methods: Protein expression of ABCA1, ABCG1 and SR-BI, and their abilities to mediate cellular cholesterol efflux were determined in human mesangial cells and tubular cells cultured under normal or high glucose conditions in vitro. Renal expression of these cholesterol transporters was examined in mice with streptozotocin-induced type 1 diabetes.

Results: ABCA1, ABCG1, and SR-BI were expressed in both human mesangial and tubular cells and were able to mediate cellular cholesterol efflux to high-density lipoprotein and apolipoprotein AI. In vitro, hyperglycaemia significantly reduced the expression of all three cholesterol transporters in a dose-dependent manner. In-vivo studies showed that renal cholesterol and triglyceride content was increased in DN mice compared to control mice. This was associated with a significant reduction in the protein expression of ABCA1, ABCG1, and SR-BI ($P < 0.05$) in the kidneys. Glomerular abnormalities and tubulo-interstitial changes were noted in DN mice and immunohistological examination showed that there was a marked reduction in all three cholesterol transporters, particularly in the renal tubules.

Conclusion: Reduced renal expression of ABCA1, ABCG1, and SR-BI was associated with DN. Defects in cholesterol export pathway in renal cells could promote cholesterol accumulation and might contribute to the development of DN.

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The role of hypothalamic APPL2 in regulation of energy metabolism

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Introduction: We have previously demonstrated that APPL1 maintains glucose homeostasis by promoting both actions and secretion of insulin. Although APPL2 is a close homologue of APPL1, the physiological function of APPL2 is vaguely characterised. Thus, we generated a knockout (KO) mouse model in which APPL2 is deleted in pancreatic β-cells and hypothalamus (which is called APPL2 KO mice) to investigate its role in glucose and energy metabolism.

Methods: Male APPL2 KO mice and their wild-type (Wt) littermates were fed with standard chow for 24 weeks. Basic metabolic parameters related to glucose and energy metabolism were examined.

Results: APPL2 KO mice displayed impairment of insulin secretion when compared to its Wt controls. Apart from the β-cell phenotypes, APPL2 KO mice displayed increased adiposity accompanied by a dramatic reduction of energy expenditure, despite similar food intake and locomotor activity. Acute cold challenge experiment revealed that APPL2 KO mice were cold sensitive, which was due to defective lipolytic and thermogenic programmes in adipose tissues. Such defects were associated with elevated activity of AMPK and expression of neuropeptide Y in hypothalamus, both of which were key molecules integrating hormonal and nutritional signals to regulate energy metabolism.

Conclusion: APPL2 not only modulates β-cell function but also regulates energy metabolism by controlling the hypothalamus-adipose tissue axis.

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Suppression of inflammation during adipogenic differentiation in human preadipocytes

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Introduction: Preadipocytes are present in adipose tissues throughout adult life that can differentiate into mature adipocytes in response to environmental factors. However, little is known about the secretory inflammation-associated cytokines / chemokines during adipogenic differentiation.

Methods: Human preadipocytes-subcutaneous (HPA-s) cells were cultured to 80% confluence. From preadipocytes to mature adipocytes, a total of six induction cycles were performed. Each cycle consisted of two sequential procedures from differentiation medium (for 3 days) to maintenance medium (for 2 days). Maintenance media after each cycle were collected to determine the levels of interleukin (IL)–6, IL-8, monocyte chemoattractant protein 1 (MCP-1), and adiponectin using commercial ELISA kits. Oil red staining was carried out to assess the degree of differentiation.

Results: The levels of pro-inflammatory cytokine IL-6 and chemokine IL-8 but not MCP-1 showed a significant trend of reduction in maintenance medium between each cycle ($P < 0.05$). On the other hand, the level of anti-inflammatory cytokine adiponectin showed a significant trend of elevation between each cycle ($P < 0.05$).

Conclusion: We demonstrated suppression of pro-inflammatory cytokine / chemokine and elevation of anti-inflammatory cytokine as the degree of differentiation increased in preadipocytes, suggesting a close relationship between adipogenic differentiation and inflammation.

The effects of hyperuricaemia on endothelial-dependent and independent vascular function in high cardiovascular risk patients

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Objectives: Hyperuricaemia has been shown to be associated with adverse cardiovascular outcome particularly in high-cardiovascular-risk patients and could be considered as a marker for cardiovascular risk assessment. One of the proposed mechanisms involving hyperuricaemia is the development of vascular damage. The aim of this study was to examine the role of hyperuricaemia on vascular function in patients with high cardiovascular risk.

Methods: We examined the relationship between hyperuricaemia and vasomotor response of the brachial artery by using high-resolution ultrasound in 304 subjects with established coronary artery disease and / or diabetes.

Results: No significant difference was observed in flow-mediated dilatation (FMD), a marker for endothelial-dependent vascular function, between the hyperuricaemic and normouricaemic group ($3.78 \pm 3.0\%$ vs $3.88 \pm 2.9\%$; $P = 0.78$). On the other hand, nitroglycerin-mediated dilatation (NMD), a marker for endothelial-independent vascular function, was significantly lower in the hyperuricaemic group compared with the normouricaemic group ($12.8 \pm 6.9\%$ vs $16.2 \pm 7.7\%$; $P < 0.001$). Multivariate analysis demonstrated that smoking was the strongest predictor of FMD ($b = –0.81$, $P = 0.02$); and that smoking ($b = –2.62$, $P = 0.003$), systolic blood pressure ($b = –0.11$, $P=0.001$), hyperuricaemia ($b = –2.11$, $P=0.02$), and use of nitrates ($b = –3.30$, $P=0.001$) were independent predictors of NMD.

Conclusion: High cardiovascular-risk patients with hyperuricaemia had a lower NMD than those with normouricaemia. Importantly, hyperuricaemia was independently associated with NMD after multivariable adjustments. To further understand the pathophysiological mechanisms involving hyperuricaemia, particularly in the context of impaired NMD, further experimental and clinical studies are needed.

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Adropin is a brain membrane-bound protein regulating physical activity via NB-3/Notch signalling pathway in mouse

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Introduction: Adropin is a highly conserved eutherian mammalian protein that was originally identified as metabolic regulator that can attenuate hepatosteatosis and hepatic insulin resistance in diet-induced obese mice. However, the physiological role of adropin and the molecular mechanism underlying its actions remains elusive. In addition, brain is the most adropin-rich organ in mouse; the role of adropin in brain has not been investigated.

Methods: Firstly, cellular fractionation was performed to determine the subcellular distribution of adropin. Secondly, yeast two-hybrid (Y2H) screening was conducted to explore the function of adropin via its binding proteins. Finally, a series of behavioural tests were performed to characterise the phenotypic changes of adropin knockout (adrKO) mice.

Results: In sharp contrast to previous reports, we found that adropin in fact is a brain-specific plasma membrane protein, instead of hepatic secretory protein. By the Y2H screening, we found that adropin binds to the brain-specific Notch1 ligand NB-3 and actives notch signalling pathway. Consistent with our findings from in-vitro experiments, adrKO mice exhibited motor coordination impairment. Same phenotype was reported in the nb-3 knockout mice.

Conclusion: Our data suggested that adropin cooperates with NB-3 mediated notch signalling in cerebellar development and function.

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Protein overload induces renal proximal tubular epithelial cell apoptosis by down-regulating Wnt/beta-catenin signalling

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Background: Numerous studies have demonstrated a tubulotoxic role of excess proteins on renal proximal tubular epithelial cell (PTECs) via various signalling pathways. However, the role of Wnt/beta-catenin signalling in PTECs during protein overload remains unknown.

Methods: Wnt/beta-catenin expressions were measured in control and human serum albumin (HSA)–treated human kidney 2 (HK-2) cells by real-time polymerase chain reaction (PCR) and Western blotting. Genetic knockdown of beta-catenin was achieved using siRNA transfection. Apoptotic phenotypes were evaluated by real-time PCR and TUNEL assay.

Results: Upon the 4-day HSA stimulation, gene expression of beta-catenin, frizzled-receptor 1, and Wnt-1 in PTECs declined by 26% ± 2 ($P < 0.05$, *t*-test), 65% ± 2 ($P < 0.05$), and 57% ± 6 ($P < 0.05$) versus control, respectively. Western blots showed that protein expression of cytosolic and nuclear active beta-catenin decreased by 57% ± 8 ($P < 0.05$) and 66% ± 8 ($P < 0.05$) after 4-day HSA treatment, respectively. Simultaneously, Bax/Bcl-2 gene expression ratio increased by 31% ± 8 ($P < 0.05$). Transfection of beta-catenin siRNA into HK-2 cells up-regulated Bax/Bcl-2 gene expression ratio by 23% ± 7 ($P < 0.05$) relative to mock transfection. HSA treatment and beta-catenin siRNA transfection increased the number of TUNEL-positive cells by 70% ± 10 ($P < 0.05$) and 73% ± 8 ($P < 0.05$), respectively.

Conclusions: Protein overload promotes tubular cell apoptosis via down-regulation of Wnt/beta-catenin signalling in PTECs.

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Clinical and cardiovascular risk profiles of type 2 diabetes and pre-diabetes patients diagnosed by HbA1c criteria

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Introduction: The objective of this study was to examine the detection rate of diabetes (DM) and prediabetes (pre-DM) based on HbA1c or glucose criteria. The cardiovascular risk profiles of the group diagnosed only by HbA1c criteria (A1c-only group) were compared to the group detected by glucose criteria (glucose group).

Methods: Participants returned for the fourth visit of the population-based Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS-4) were included in this study. Detailed medical histories, anthropometric measurements, and assessments of cardiovascular risk factors were performed in 1584 subjects. All non-diabetic participants (n = 1413) had glycaemic status assessed by 75-g oral glucose tolerance test (OGTT) and HbA1c. DM was defined by HbA1c of ≥ 6.5%, fasting glucose (FG) of ≥ 7.0 mmol/L or 2-hr post-OGTT glucose (2hG) of ≥ 11.1 mmol/L. Subjects with HbA1c 5.7-6.4%, FG 5.6-6.9 mmol/L, or 2hG 7.8-11.0 mmol/L were considered pre-DM.

Results: A total of 274 (17.3%) subjects had DM, 171 (10.8%) were known and 103 (6.5%) were newly diagnosed. Another 860 subjects (54.3%) were found to have pre-DM. Detection rate of DM and pre-DM were 11.7%, 11.5% (FG); 44.7%, 20.8% (2hG); and 80.6%, 95.5% (HbA1c), respectively. For the newly diagnosed DM subjects, the A1c-only group had significantly lower HOMA-IR, triglycerides but higher HDL than the glucose group. For the pre-DM subjects, the A1c-only group were younger (58.3 ± 9.77 vs 60.6 ± 10.3 years; P = 0.002), less obese (body mass index: 23.8 ± 3.24 vs 25.0 ± 3.61 kg/m^2; P < 0.001; waist circumference: 81.0 ± 9.14 vs 85.1 ± 9.35 cm; P < 0.001), less hypertensive (30.7% vs 55.2%; P < 0.001), less insulin-resistant (HOMA-IR: 1.39 vs 2.03; P < 0.001), and less dyslipidaemic (higher HDL and lower triglycerides, P = 0.005 and < 0.001) comparing to the glucose group. The aforementioned parameters were more adverse in the A1c-only group when compared with normal subjects.

Conclusion: The application of HbA1c criteria detects a large population of DM and pre-DM subjects in the Chinese population. The A1c-only group appears to have more favourable risk profiles than the glucose group. Whether this predicts fewer cardiovascular events requires further examination by longitudinal studies. Nevertheless, early identification of such an at-risk population, which could be missed by the glucose criteria, should help to control the rapidly growing diabetic population in China with the implementation of proper preventive measures.

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Primary aldosteronism: identifying suitable cases for confirmatory test

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Introduction: Primary aldosteronism (PA) is a potentially curable form of secondary hypertension and is believed to be under-diagnosed. However, performing confirmatory test on every patient with suspected PA is not practical. The aim of the study was to identify the most appropriate screening test(s) and define their cut-off value(s).

Methods: Patients with suspected PA were investigated according to a standardised protocol. Saline infusion test was done and patients were considered unlikely to have PA (PA unlikely) if the post-infusion aldosterone level result was less than 140 pmol/L. Subjects with results higher than this were considered as 'PA possible'. Baseline characteristics of patients and biochemical measurements were compared. Area under receiver operating characteristic curves (AROC) was used to examine the discriminative ability of individual parameter for prediction of 'PA possible' cases. Sensitivity, specificity, negative and positive predictive values were calculated according to different cut-offs of individual parameters, as well as their combinations.

Results: Of the 99 subjects, 21 were considered 'PA possible'. Aldosterone-to-renin ratio (ARR) [1474 (interquartile range, 724.9-2702) vs 461.9 (306.7-740.9); P < 0.001], aldosterone (PAC) [471 pmol/L (309-733) vs 265 pmol/L (169-365); P < 0.001], and plasma renin activity (PRA) [0.30 ng/mL/h (0.20-0.60) vs 0.50 ng/mL/h (0.30-1.60); P = 0.003] were significantly different between the 'PA possible' and 'PA unlikely' groups. Receiver operating characteristic curves analysis showed that ARR (AROC, 0.81) and PAC (AROC, 0.78) showed better discriminative performance than PRA (AROC, 0.69). The ARR model with cut-off at 400 yielded a sensitivity of 85.9% and specificity of 42.9%. Addition of PAC with cut-off value at 200 pmol/L to the ARR model improved the specificity to 57.1% with only a slight reduction of sensitivity to 83.3%.

Conclusion: ARR and PAC have good predictive performance for cases with possible PA. Cut-off values are derived to achieve a good sensitivity and fair specificity for clinical use as a reliable screening test.

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Visual analogue scale for back pain predicts osteoporotic fracture in a southern Chinese population

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Introduction: The aim of this study was to assess the fracture incidence in a southern Chinese population with low bone mass and examine if there is an association between a visual analogue scale (VAS) for back pain and development of osteoporotic fracture in the population.

Methods: A total of 1732 southern Chinese subjects referred for management of low bone mineral density (BMD) were recruited for analysis. Systematic assessments were performed in the first visit with a 10-point VAS back pain score rated by a nurse. The occurrence of incident osteoporotic fracture was determined yearly by subjects' self-reports and data retrieved from the hospital's Computer Medical System. A cox proportional hazards regression model was used to determine the association between VAS for back pain and major osteoporotic fractures, with adjustments made for age, BMD of hip and spine, prevalent fracture, and gender.

Results: A total of 979 female (56%) and 753 male (44%) were included in the study. The mean age of the subject was 63 ± 9 years and median duration of follow-up was 3 (interquartile range, 1-4) years. Among the study subjects, 70 cases (4.1%) developed new low-trauma osteoporotic fracture (spine: 24, 1.4%; hip: 10, 0.6%; distal radius: 8, 0.5%; others: 28, 1.6%). The mean VAS for back pain was higher among subjects with incident fracture (5 ± 4) than those without (2 ± 2) [$P < 0.001$]. Furthermore, a high VAS for back pain was significantly associated with the development of new osteoporotic fracture even after adjustment for gender, age, total spine and total hip BMD, and particularly, the presence of prevalent fracture (hazard ratio [HR] = 1.34; 95% confidence interval [CI], 1.25-1.44; $P < 0.001$). A VAS rating of ≥9 was associated with a high risk of sustaining a new osteoporotic fracture (HR = 11.97; 95% CI, 7.09-19.86; $P < 0.001$).

Conclusion: VAS of back pain is an independent predictor for incident osteoporotic fracture in our southern Chinese population and has the potential to serve as a simple and non-invasive clinical tool to further complement our existing methods for fracture risk prediction.

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Mesenchymal stem cells attenuate albumin-induced tubulointerstitial fibrosis

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Introduction: Tubulointerstitial fibrosis is a key manifestation of chronic kidney disease (CKD). Mesenchymal stem cells (MSCs) have recently shown protective effects in CKD. The mechanism of action, however, is not completely defined. This study aimed to explore the potential anti-fibrotic impact of MSCs on protein-overloaded proximal tubular epithelial cells (PTECs) and proteinuric mice.

Method: In-vitro human PTECs were treated with human albumin serum and co-cultured with MSCs. Tubular epithelial-to-mesenchymal transition (EMT) was assessed by detecting the expression of epithelial and mesenchymal markers. In-vivo albumin-overloaded mice received multiple MSCs injection. Renal fibrosis was examined by deposition of extracellular matrix proteins in the tubulointerstitium.

Results: In albumin-overloaded PTECs, MSCs significantly prevented the downregulation of E-cadherin mRNA, and reduced overexpression of α-SMA, collagen IV and fibronectin mRNA. The loss of E-cadherin protein and overexpression of collagen IV protein in PTECs were also rescued by MSC co-culture. In-vivo albumin-overloaded mice treated with mouse MSCs had markedly reduced α-SMA and collagen IV accumulation in the tubulointerstitium together with reduced blood urea nitrogen independent of changes in proteinuria.

Conclusion: We conclude that MSCs exerted anti-EMT effect in albumin-overloaded PTECs, which are translated to an anti-fibrotic action in the protein-overload nephropathy model reminiscent of human CKD.

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Association of physical activity with cognitive function, behavioural symptoms, and caregiver's burden in Chinese dementia patients

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Introduction: Dementia patients show impairments of memory and other cognitive functions with consequent decline in activities of daily living. Besides cognitive symptoms, demented patients can also exhibit behavioural and psychological symptoms of dementia, which are stressors leading to family caregivers' burden. Physical activity may give rise to benefits in cognitive function, and may reduce behavioural symptoms and caregivers' burden. The objective of this study was to investigate the associations of physical activity level with cognitive function, behavioural and psychological symptoms and caregivers' burden in Chinese dementia patients in Hong Kong.

Methods: This was a cross-sectional study. A total of 201 dementia patients were recruited from the Geriatric Clinic in Queen Mary Hospital from May 2013 to August 2013. Data on demographic, comorbid diseases, cognitive function (Mini-Mental State Examination [MMSE] and Montreal Cognitive Assessment [MoCA]), physical function (Alzheimer's Disease Cooperative Study Activities of Daily Living Inventory [ADCS-ADL]), physical activity (Physical Activity Scale for the Elderly [PASE]), and behavioural symptoms (Neuropsychiatric Inventory [NPI]) were collected. Burden of the subjects' family caregivers were assessed with the Zarit Burden Interview (ZBI).

Results: A total of 201 subjects (70 males and 131 females) were recruited. In bivariate analysis, the PASE score was significantly associated with the MMSE score (rho = 0.259, $P < 0.001$), the MoCA score (rho = 0.311; $P < 0.001$), the NPI score (rho = –0.225, $P = 0.001$), and the ZBI score (rho = –0.253, $P < 0.001$). In multivariate analyses, using general linear models, the PASE score was independently associated with the MMSE score ($F = 5.57$, $P = 0.001$), the MoCA score ($F = 7.10$, $P < 0.001$), and the NPI score ($F = 2.89$, $P = 0.037$) after adjusting for significant confounders in univariate analyses. The subjects' ADCS-ADL score ($F = 15.65$, $P < 0.001$) and the NPI score ($F = 8.55$, $P = 0.004$) were independent predictors of the caregiver's ZBI score.

Conclusion: Physical activity is associated with improvements in both cognitive function, and behavioural and psychological symptoms among Chinese dementia patients in Hong Kong. However, there is no relationship between patients' physical activity level and their family caregivers' burden.

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Visit-to-visit blood pressure variability is associated with periventricular white matter hyperintensity in healthy hypertensive elderly Chinese

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Background: Visit-to-visit blood pressure variability (BPV) has been reported to be associated with the severity and prognosis of symptomatic stroke, but its correlation with silent stroke is unclear. We aimed to evaluate the relationship between BPV and silent cerebral vascular lesions (SCVL).

Methods: A total of 239 healthy hypertensive Chinese aged more than 65 years were recruited. SCVLs including silent brain infarcts (BIs), microbleeds (MBs), and white matter hyperintensity (WMH) were determined by magnetic resonance images on a 3T scanner. The severity of WMH was rated using the Fazekas white matter scale. Previous 3-year blood pressure measurements were collected from the electronic medical record. BPV parameters—including standard deviation, coefficient of variation, successive variation, and variation independent of mean blood pressure—were calculated. Statistical analyses were performed to evaluate the significance of relationships.

Result: Of the patients, 26 (10.9%) and 12 (5.0%) had severe periventricular WMH and deep WMH (Fazekas score = 3), respectively. Both systolic and diastolic BPV parameters were correlated with the severity of periventricular WMH ($P < 0.05$) but not the severity of deep WMH, presence of BIs or MBs. The relationship between BPV parameters and periventricular WMH still existed after adjustment for age and other factors.

Conclusion: BPV was an independent predictor of severe periventricular WMH in healthy hypertensive elderly.

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MicroRNA-mediated mechanisms in hyperactive plasmacytoid dendritic cells in systemic lupus erythematosus

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Introduction: Systemic lupus erythematosus (SLE) patients are typically presented by their increased serum type I interferon (IFN) activities. Plasmacytoid dendritic cells (pDCs), the most potent type I IFN-producing cells, are found to be hyperactive in SLE. Previous studies have found consistently higher expression of microRNA-155 (miR155) in lupus pDCs upon TLR7 stimulation. Therefore, using the New Zealand Black/White F1 lupus mouse model, the current investigation pursued on the correlation between the up-regulated miR155 induction and aberrant pDC functions in SLE upon microRNA transfection.

Methods: Mice with lupus symptoms such as high titres of serum anti-nuclear antibodies and persistent proteinuria were compared with the pre-symptomatic ones. MiR155 mimics or scrambled control microRNAs were transfected into pre-symptomatic pDCs and subsequently the induction of responsive genes were quantified upon TLR7 stimulation.

Results: An increase basal expression of *Tlr7* was found in the symptomatic pDCs. Consistently, upregulated induction of the IFN-stimulated gene, *Ifitm3* was found in lupus pDC in response to TLR7 stimulation. On the other hand, pre-symptomatic pDCs, upon miR155 transfection, lead to a reduced pDC response to TLR7 activation. Decreased induction of *Tnf-alpha* and *Ifitm3*, was found in miR155-transfected pDCs when compared with scrambled controls.

Conclusion: It was previously found in human studies that miRNA-155 negatively regulates type I IFN production by pDCs and itself inversely regulated by the autocrine/paracrine IFN dynamics. However, the mechanism of the association between miR155 expression and increased IFN response in SLE requires further investigations.

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Functional abnormalities and regulatory mechanisms of plasmacytoid dendritic cells in the development of systemic lupus erythematosus

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Introduction: Systemic lupus erythematosus (SLE) is a chronic multi-organ autoimmune disease with immunological features of a prominent 'interferon (IFN) signature', which is marked by the elevated expression of type I IFN-regulated genes in blood and tissue cells of patients with this condition. Plasmacytoid dendritic cells (pDCs), the most potent type I IFN-producing cells, are previously found to be hyperactive in SLE. Using the New Zealand Black/White F1 lupus mouse model, the current study sought for the regulatory mechanism of IFN production by pDCs in SLE.

Methods: Mice with lupus symptoms (symptomatic) such as high titres of serum anti-nuclear antibodies and persistent proteinuria were compared with the pre-symptomatic ones. Age- and sex-matched non-lupus maternal NZW mice were used as controls.

Results: While the development of pDCs appeared to be unaffected by lupus, elevated upregulation of MHC class II and co-stimulatory molecules, and induction of IFN-stimulated gene *Ifitm3* in TLR7-stimulated lupus pDCs suggested phenotypic and functional hypersensitivity of these cells. Furthermore, analysis of the expression profile of microRNAs in pDCs upon TLR7 activation identified six differentially regulated targets. Among these, miR-155 was the most highly induced and its induction was consistently higher in pDCs from symptomatic NZB/W F1 mice.

Conclusion: Results in the current study demonstrated that pDCs in SLE exhibited heightened responses to TLR7 activation, which is possibly associated with miR-155-mediated pDC functional dysregulation. It is hoped that findings from this study contribute to a better understanding of SLE pathogenesis and ignite future interests in evaluating the molecular layer of regulation in autoimmunity.

Preliminary efficacy, safety, pharmacokinetics, pharmacodynamics and quality of life study of pegylated recombinant human arginase 1 (Peg-rhArg1) treatment in patients with advanced hepatocellular carcinoma

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Background: This clinical study was designed to evaluate the efficacy, safety profile, pharmacokinetics/pharmacodynamics (PK/PD), and quality of life (QoL) of pegylated recombinant human arginase 1(Peg-rhAgr1) in patients with advanced hepatocellular carcinoma (HCC), based on previous work by our group.

Methods: Patients with advanced inoperable HCC were given weekly doses of Peg-rhAgr1 (1600 U/kg). Tumour response was assessed every 8 weeks using RECIST 1.1 and modified RECIST criteria. Disease control rate (DCR), safety profile, PK/PD, and QoL were analysed.

Results: A total of 20 patients were recruited, of whom 15 were deemed evaluable for treatment efficacy. Five had no prior treatment. Fifteen had pretreatment with at least two rounds of transarterial chemoembolisation, two of whom also had systemic biologics. Eighteen patients (90%) were hepatitis-B carriers. The median age was 61.5 (range, 30-75) years. Overall DCR was 13%, with two of the 15 patients achieving stable disease for >8 weeks. Median progression-free survival (PFS) in this group was 1.7 (95% confidence interval [CI], 1.67-1.73) months, with a median overall survival (OS) of all 20 enrolled patients being 5.2 (95% CI, 3.3-12.0) months. PFS was significantly prolonged in patients with adequate arginine depletion (ADD) of >2 months versus those who had ≤2 months of ADD (6.4 vs 1.7 months; P = 0.01). The majority of adverse events (AEs) were grade 1 and 2 non-haematological toxicities. Transient liver dysfunctions (25%) were the most commonly reported serious AEs, probably related to disease progression. Pharmacokinetic and pharmacodynamic data show that Peg-rhAgr1 induced rapid and sustained arginine depletion as long as the drug continued to be administered. The overall QoL of the enrolled patients was well preserved.

Conclusion: Peg-rhAgr1 is well tolerated with a good toxicity profile in patients with advanced HCC. A weekly dose of 1600 U/kg is sufficient to induce ADD. Significantly longer PFS times were recorded for patients who had ADD for >2 months. A larger study is planned to explore the anti-tumour efficacy of Peg-rhArgI together with oxaliplatin-based chemotherapy and to confirm the relationship of ADD with OS and PFS.

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Fibroblast growth factor 21 protects against acetaminophen-induced hepatotoxicity by potentiating PGC-1α-mediated antioxidant capacity in mice

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Background: Acetaminophen (APAP) overdose is a leading cause of drug-induced hepatotoxicity and acute liver failure worldwide, but its pathophysiology remains incompletely understood. Fibroblast growth factor 21 (FGF21) is a hepatocyte-secreted hormone with pleiotropic effects on glucose and lipid metabolism. This study aimed to investigate the pathophysiological role of FGF21 in APAP-induced hepatotoxicity in mice.

Methods: FGF21 knockout (KO) mice and their wild type (WT) littermates were intraperitoneally injected with APAP (500 mg/kg) to induce acute liver injury. Recombinant FGF21 or adenovirus expressing either peroxisome proliferator-activated receptor coactivator protein-1α (PGC-1α) or siRNA against PGC-1α were used to treat mice to test their effects on APAP hepatotoxicity.

Results: In response to APAP overdose, both hepatic expression and circulating levels of FGF21 in WT mice were dramatically increased as early as 3 hours, prior to elevations of the liver injury markers alanine aminotransferase and aspartate aminotransferase. APAP overdose–induced liver damage and mortality in FGF21 KO mice were markedly aggravated, which was accompanied by increased oxidative stress and impaired anti-oxidant capacities as compared to WT littermates. By contrast, replenishment of recombinant FGF21 largely reversed APAP-induced hepatic oxidative stress and liver injury in FGF21 KO mice. Mechanistically, FGF21 induced hepatic expression of PGC-1α, thereby increasing the nuclear abundance of nuclear factor erythroid 2-related factor 2 (Nrf2) and subsequent upregulation of several anti-oxidant genes. The beneficial effects of recombinant FGF21 on upregulation of Nrf2 and anti-oxidant genes and alleviation of APAP-induced oxidative stress and liver injury were largely abolished by adenovirus-mediated knockdown of hepatic PGC-1α expression, whereas overexpression of PGC-1α was sufficient to counteract the increased susceptibility of FGF21 KO mice to APAP-induced hepatotoxicity.

Conclusion: The marked elevation of FGF21 by APAP overdose may represent a compensatory mechanism to protect against the drug-induced hepatotoxicity, by enhancing PGC-1α /Nrf2-mediated anti-oxidant capacity in the liver.

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Plasmacytoid dendritic cells in systemic lupus erythematous

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Introduction: Plasmacytoid dendritic cell (pDC) is a potent producer of type I interferon (IFN-I). Interestingly, one of the serological hallmarks of systemic lupus erythematous (SLE) is the up-regulation of serum IFNα which correlates to the disease severity. In addition, pDCs were found to infiltrate into inflammatory sites such as skin and kidney in SLE patients suggesting that pDC may play a role in SLE pathogenesis. Using the BWF1 SLE mouse model, this study aimed to investigate whether pDC displays any abnormality in SLE that may contribute to the disease pathogenesis.

Methods: CD11c and CD317 double-positive splenic pDCs were isolated from pre-disease and disease BWF1 by FACS-sorting. The expression level of different activation markers on pDC was analysed by flow cytometry. To compare the expression of activation makers on activated pDC between disease and pre-disease BWF1, pDC was stimulated by toll-like receptor (TLR) 7 and TLR9 ligand for 24 hours using 1μM CpG or 2.5 μg/mL R837 followed by flow cytometry.

Results: Un-stimulated splenic pDCs isolated from BWF1 did not show any difference in MHC II, CD40, CD86 expression, although CD80 expression was significantly higher in disease BWF1. Upon TLR7 and TLR9 stimulation, disease and pre-disease BWF1 pDC did not show any difference in CD40 and MHC II expression.

Conclusion: Only CD80 had displayed a higher expression on un-stimulated disease BWF1 splenic pDC. More activation markers can be analysed with different TLR 7 and 9 ligands stimulation to further investigate whether pDCs in SLE are more responsive to activation and their role in SLE pathogenesis.

Tissue kallikrein mediates pro-inflammatory pathways and activation of protease-activated receptor-4 in proximal tubular epithelial cells

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Introduction: Tissue kallikrein (KLK1) expression is up-regulated in human diabetic kidney tissue. Since the kallikrein-kinin system (KKS) has been linked to cellular inflammatory process in many diseases, we explored the role of KLK1 in tubular pro-inflammatory responses under the diabetic milieu.

Methods: The expression of pro-inflammatory cytokines and activation of signalling pathway were determined after treatment of recombinant KLK1 to human proximal tubular epithelial cells (PTEC). The effect of KLK1 on advanced glycation end products (AGE)–induced tubular inflammation was examined by siRNA gene silencing. Activation of protease-activated receptors (PARs) by KLK1 was studied by calcium mobilisation assay.

Results: Recombinant KLK1 stimulated the production of inflammatory cytokines including IL-8, ICAM-1 and CCL-2, and activated the phosphorylation of p42/44 and p38 MAPK signaling in PTEC. Increased expression of KLK1 was detected in PTEC stimulated with AGE, and molecular knockdown of endogenous KLK1 expression attenuated AGE-induced tubular IL-8 and ICAM-1 productions. Furthermore, KLK1 stimulated the activation of protease-activated receptor-4 (PAR-4), while blockade of PAR-4 by antagonist attenuated the induced pro-inflammatory cytokine production.

Conclusion: Our data demonstrate that KLK1 mediates pro-inflammatory responses in renal tubule cells via PAR-4 activation, and provide a new therapeutic target for diabetic tubular injury.

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Absence of association between arterial stiffness and white matter hyperintensities in otherwise healthy hypertensive elderly Chinese

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Introduction: Arterial stiffness has been observed to be associated with white matter hyperintensities (WMH) in different populations. This study aimed to investigate whether such association exists in otherwise healthy hypertensive elderly Chinese.

Methods: Degree of WMH has been assessed based on fluid-attenuated inversion recovery (FLAIR) magnetic resonance imaging using Fazekas white matter scale scores, and then dichotomised to compare with quartiles of brachial-ankle pulse wave velocity (BaPWV), a marker of arterial stiffness. Univariate and multivariate logistic regression models were used to determine the odds ratio (OR) for advanced WMH.

Results: We studied a population of 252 otherwise healthy hypertensive Chinese over 65 years old, and advanced WMH was present in 53 (22.4%). In the highest BaPWV quartile, 22 (34.9%) subjects had advanced WMH. Significant association was observed between the highest quartile of BaPWV and advanced WMH when using the univariate logistic regression model. However, after controlling for demographic and arterial vascular risk factors (age, gender, body mass index, smoke history, grade of hypertension, duration of hypertension, total cholesterol, triglycerides, and high-density lipoprotein cholesterol), there was no statistically significant association (odds ratio = 2.101; 95% confidence interval, 0.781-5.649).

Conclusion: This study did not provide any evidence for significant association between arterial stiffness and WMH. Fazekas white matter scale score may be too simple a tool to reveal the association and we shall pursuit more suitable resolution for further investigation.

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Relationship between diabetic retinopathy and subclinical myocardial dysfunction in patients with diabetic mellitus

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Background: Diabetic mellitus (DM) patients may have cardiac structure and functional changes or microvascular disease in the absence of cardiovascular disease history. The relationship between the occurrence of diabetic microvascular disease and cardiac change in DM patients without a history of heart trouble is unclear. The present study sought the correlation between diabetic retinopathy, which is one kind of microvascular disease, and cardiac functional changes by (a) basic ophthalmic testing and (b) detailed echocardiography for cardiac assessment.

Methods: A total of 251 patients with type 2 DM without cardiovascular diseases were recruited. Transthoracic echocardiography was performed at the rest state as well as after treadmill exercise and analysed in detail with the following parameters: (i) left ventricle (LV) systolic function was assessed by Simpson's method derived ejection fraction (EF) and speckle tracking derived global longitudinal strain (GLS), (ii) myocardial structural alteration by calibrated integrated backscatter (cIBS), (iii) diastolic function by tissue Doppler derived E/E' ratio, and (iv) diastolic function reserve index (DFRI). Furthermore, all patients undertook a full-fledged photography programme. Retinopathy was scored and classified as with retinopathy or without diabetic retinopathy.

Results: Of the 251 subjects (mean age, 63.13 ± 9.31 years; 46.2% male), 24.3% had retinopathy. Patients with and without diabetic retinopathy (as a categorical variable) had similar LVEF and cIBS. However, retinopathy had a significant correlation with LV GLS ($r = –0.18$, $P < 0.01$), E/E' ($r = 0.19$, $P < 0.01$), diastolic dysfunction grade ($r = 0.18$, $P < 0.01$), and DFRI ($r = –0.23$, $P = 0.02$). Furthermore, these correlations were independent of potential confounding factors.

Conclusion: This study indicated that the occurrence of retinopathy significantly correlates with GLS, E/E', diastolic dysfunction grade, and DFRI. Above all, the correlation still existed after adjusted by potential confounding factors, suggestive of an independent relation between retinopathy and cardiac function. The link between these parameters highlighted the importance of further cardiac assessment and timely treatment for DM patients with diabetic retinopathy who have no cardiac disease history and relevant symptoms.

Combination of arsenic trioxide and chemotherapy in small-cell lung cancer

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Introduction: Small-cell lung cancer (SCLC) carries a high mortality despite standard chemotherapy. Arsenic trioxide (ATO) has demonstrated clinical efficacy in leukaemia and in-vitro activity in various solid tumours. This study was conducted to determine the in-vitro and in-vivo combination effects of ATO and chemotherapy in SCLC.

Methods: The in-vitro model consisted of five SCLC cell lines and the anti-proliferative effects of ATO, cisplatin, etoposide or combinations thereof were measured. Assays for apoptosis, intracellular glutathione (GSH) content, and mitochondrial membrane depolarisation (MMD) were performed. Arsenic content was measured by inductively coupled plasma-mass spectrometry. Expression level of MRP1, MRP2 and pH2AX was detected by Western blot while cellular pH2AX level was monitored by immunofluorescent staining. An in-vivo xenograft model in nude mice was established with a H841 cell line to test the effects of drug combinations.

Results: All five SCLC cell lines were sensitive to ATO, with IC50 values (48h) 1.6-8 μM. Synergistic or additive effects were obtained by combining cisplatin with ATO in all five cell lines. Combination of etoposide with ATO resulted in antagonistic or barely additive effects. Apoptotic assays and pH2AX immunofluorescent staining corroborated the synergistic combination of ATO and cisplatin. In addition, the ATO/cisplatin combination enhanced MMD, depleted GSH, downregulated MRP2, and elevated intracellular ATO content compared with either ATO or cisplatin alone. In-vivo combination of ATO and cisplatin also demonstrated synergism in the H841 xenograft model.

Conclusions: There was clinically relevant in-vitro activity of ATO in a panel of five SCLC cell lines. Significant synergism was demonstrated with the ATO/cisplatin combination, while antagonism was noted with the ATO/etoposide combination in both in-vitro and in-vivo models.

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