Plenary 1

From epithelial cells to body function and disease—with particular focus on the reproductive system

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Epithelial cells line the lumens of many vital organs, including the lung, pancreas, intestine, and the reproductive tracts. Epithelial cell functions, particularly epithelial secretions, are important to various organ functions, defect of which may lead to various pathological conditions as simple as diarrhoea and as lethal as cystic fibrosis and cancer. This talk will provide an overview of our work on epithelial cell–related interdisciplinary research with particular focus on the reproductive system.

The lumens of the male and female reproductive tracts have epithelial linings with secretions that are thought to be important for various reproductive events. Our recent research aims to elucidate how these reproductive events are regulated by epithelial secretions of both reproductive tracts. Our results show that epithelial secretions of the testis, epididymis, uterus, and oviduct play important roles in regulating the process of spermatogenesis, sperm maturation, capacitation, and embryo development. Defects in epithelial cell function could lead to pathological conditions and affect fertility outcome.

Plenary 2

eNOS, endothelial dysfunction, and cardiovascular disease

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Large epidemiologic studies have established that type 2 diabetes, atherogenic dyslipidemia, hypertension, and obesity all increase the risk for cardiovascular disease. However, the precise mechanisms by which these metabolic disorders increase the propensity to develop atherosclerosis and cardiovascular disease are not known. Recently, the concept of the metabolic syndrome, a constellation of conditions including obesity, hypertension, hyperlipidemia, and insulin resistance, has received much attention. Although the concept is controversial, the concept of the metabolic syndrome identifies a subgroup of patients with shared pathophysiology who are at high risk of developing cardiovascular disease and type 2 diabetes. By considering the central features of the metabolic syndrome, and how they are related, we may better understand the underlying pathophysiology and disease pathogenesis.

The central features of the metabolic syndrome are visceral adiposity (which leads to increased FFA, adipokines, and cytokines), atherogenic dyslipidemia (with low HDL, high triglyceride levels, and increase in small, dense LDL containing apoB), insulin resistance (with resulting hyperglycemia, as well as glucose-independent effects), and endothelial dysfunction, leading to vasoconstriction, hypertension, and decreased NO generation). eNOS and NO are critically important to cardiovascular disease, because they serve as a final common pathway by which cardiovascular risk factors are translated into effects on the vessel wall, by effects on vascular tone, leukocyte-endothelial interactions and inflammation, and platelet aggregation and adhesion. In addition, new evidence suggests that eNOS and NO may play primary roles in energy metabolism and bioenergetics.

This broad framework of atherogenesis provides a detailed understanding of the complex interrelationships between individual risk factors and may lead to new insights into pharmacologic and lifestyle treatment approaches for cardiovascular disease.

A single-centre cross-sectional study of liver stiffness in patients with primary biliary cirrhosis in Hong Kong

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Introduction: Liver stiffness assessed by transient elastography was found to correlate with fibrosis stage in patients with chronic viral hepatitis as well as in western and Japanese patients with primary biliary cirrhosis (PBC). This study investigated for the liver stiffness measurements and disease prognostic factors in Chinese patients with PBC.

Methods: Sixty Chinese patients diagnosed with PBC attending the Department of Medicine in Queen Mary Hospital were recruited for liver stiffness measurement by Fibroscan. Blood tests were taken within 1 week of the liver stiffness measurement. Demographic data, symptoms, medication use, history of liver decompensation were reviewed. Blood test results at the time of disease presentation, before and 1 year after the commencement of ursodeoxycholic acid were retrieved. Prognostic scores were calculated at disease presentation and within 1 week of the liver stiffness measurement.

Results: Of the 60 PBC patients, 53 had a valid liver stiffness measurements obtained at a median of 48 months after their first disease presentation; 21 patients had decompensated liver cirrhosis. The median values of Mayo risk score, MELD score and liver stiffness were 4.5, 5.2, and 11.8 kPa, respectively. The liver stiffness measurements correlated with WCC, haemoglobin, platelet count, prothrombin time, serum albumin, bilirubin, globulin, and AST. It also correlated well with predictive scores such as Mayo risk score and MELD score.

Conclusion: A higher liver stiffness measurement was associated with clinical cirrhotic features, advance blood test results, and worse survival prognostic scores. The measurement may be useful in Chinese PBC patients to predict, quantify, and monitor liver fibrosis.

Rehabilitation outcomes of Chinese patients with different cognitive function in geriatric day hospital

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Introduction: The effect of cognition on rehabilitation outcomes has been controversial. This study was conducted to examine the effect of cognition on rehabilitation outcomes in older patients undergoing geriatric day hospital (GDH) training.

Methods: It was a retrospective study performed in the GDH of Fung Yiu King Hospital. Cognitive status was assessed with Cantonese version of Mini-Mental State Examination (C-MMSE). Patients were stratified into three C-MMSE groups: <10, ≥10-19, and ≥20. Functional Independence Measure (FIM) upon GDH admission (FIM admission) and discharge (FIM discharge) was measured. FIM efficacy was FIM discharge – FIM admission, while FIM efficiency was FIM efficacy divided by number of GDH visits. FIM discharge ≥90 was defined as satisfactory outcome of rehabilitation.

Results: A total of 547 patients attended GDH between January 2005 and December 2007 were studied. A significant positive correlation was observed between C-MMSE admission and FIM discharge (P<0.001). There were significant differences in the FIM admission and FIM discharge among the three C-MMSE groups, with lower discharge scores in low C-MMSE groups (P<0.001). The FIM efficacy and FIM efficiency during GDH rehabilitation were not different among different C-MMSE groups. Multivariate analysis showed that C-MMSE admission (odds ratio=1.08; 95% CI, 1.0-1.15; P=0.03) and FIM admission (odds ratio=1.33; 95% CI, 1.25-1.41; P<0.001) were both positive independent predictors for a satisfactory rehabilitation outcome (FIM discharge ≥ 90).

Conclusion: In GDH, patients with poor cognition had lower FIM discharge. Cognitive function was an independent predictor for satisfactory rehabilitation outcome. However, cognitive function was not associated with FIM efficacy and efficiency. This suggested that selected patients with impaired cognition could still benefit from GDH rehabilitation.

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Label-free separation of human embryonic stem cells and their cardiac derivatives using Raman spectroscopy

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Self-renewable, pluripotent human embryonic stem cells (hESCs) can be differentiated into cardiomyocytes (CMs), providing an unlimited source of cells for transplantation therapies. However, unlike certain cell lineages such as haematopoietic cells, CMs lack specific surface markers for convenient identification, physical separation, and enrichment. Identification by immunostaining of cardiac-specific proteins such as troponin requires permeabilisation, which renders the cells unviable and non-recoverable. Ectopic expression of a reporter protein under the transcriptional control of a heart-specific promoter for identifying hESC-derived CMs (hESC-CMs) is useful for research but complicates potential clinical applications. The practical detection and removal of undifferentiated hESCs in a graft, which may lead to tumours, is also critical. Here, we demonstrate a non-destructive, label-free optical method based on Raman scattering to interrogate the intrinsic biochemical signatures of individual hESCs and their cardiac derivatives, allowing cells to be identified and classified. By combination of the Raman spectroscopic data with multivariate statistical analysis, our results indicate that hESCs, human foetal left ventricular CMs, and hESC-CMs can be identified by their intrinsic biochemical characteristics with an accuracy of 96%, 98%, and 66%, respectively. The present study lays the groundwork for developing a systematic and automated method for the non-invasive and label-free sorting of (1) high-quality hESCs for expansion, and (2) ex-vivo CMs (derived from embryonic or adult stem cells) for cell-based heart therapies.

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Elevated plasma adiponectin levels in patients with chronic obstructive pulmonary disease

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Introduction: Adiponectin is an anti-inflammatory adipokine and is thought to play a role in chronic obstructive pulmonary disease (COPD) pathogenesis. This study was to investigate plasma levels of adiponectin, interleukin (IL)-8, and C-reactive protein (CRP) in ever-smokers with or without COPD.

Methods: Plasma levels of adiponectin, IL-8, and CRP were measured using commercially available kits respectively in COPD patients (n=71), healthy ever-smokers (n=62), and non-smokers (n=51). Pulmonary function test was also carried out for all subjects recruited in this study.

Results: There were significant increases in plasma adiponectin and CRP in COPD patients (median [IQR], 4.39 μg/mL [2.68-6.98 μg/mL] and 8.75 mg/L [4.26-40.63 mg/L] respectively) compared to healthy ever-smokers (1.90 μg/mL [0.86-2.86 μg/mL] and 3.71 mg/L [1.97-10.37 mg/L] respectively; P<0.001) and non-smokers (1.76 μg/mL [1.34-2.52 μg/mL] and 3.12 mg/L [2.11-5.71 mg/L] respectively; P<0.001). Patients with COPD, however, had a lower plasma IL-8 than healthy ever-smokers. Plasma adiponectin and CRP increased with COPD severity while IL-8 was reduced. Among ever-smokers with or without COPD, plasma adiponectin and CRP levels were inversely correlated with FEV_1 (% predicted) after adjustment for age, body mass index, smoking status, and pack-years smoked.

Conclusion: Plasma adiponectin levels are associated with disease severity in COPD patients, suggesting a possible role in the pathogenesis of COPD.

Facial depigmentation associated with low-fluence Q-switched 1064-nm Nd:YAG laser for skin rejuvenation and melasma

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Background: 'Laser toning', defined by low-fluence, large spot size, multiple passed Q-switched 1064-nm Nd:YAG laser, has gained much popularity in Asian countries for non-ablative skin rejuvenation and the treatment of melasma. One of the complications associated with laser toning is facial depigmentation.

Methods: Fourteen patients with laser toning–associated facial depigmentation were assessed with cross-polarised and ultraviolet (UV) photographic images captured with the Canfield Visia CR system. The laser toning regimens received by these patients, as well as the treatment given for depigmentation, were analysed retrospectively.

Results: All 14 patients were Chinese females, eight of which received laser toning for non-ablative skin rejuvenation and the others for melasma. The treatment regimens received by these patients were highly variable. The total number of treatments received ranged from 6 to 50 (mean, 22). Ultraviolet photographic images demonstrated facial mottled depigmentation in all patients. Laser toning failed to significantly improve the melasma in five patients. Four patients received targeted NB-UVB for treatment of depigmentation with good clinical results.

Conclusion: Laser toning with low-fluence Q-switched 1064-nm Nd:YAG laser for skin rejuvenation and melasma can be associated with mottled depigmentation. With laser toning being frequently performed, this complication may become more commonly encountered in clinical practice. The depigmentation can appear after only a few treatment sessions, and can cause much disfigurement, especially in cases with background melasma. Further studies on laser toning are needed with the view to optimising efficacy and minimising side-effects.

The use of non-ablative fractional resurfacing in Asian acne scar patients

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Background: Non-ablative fractional resurfacing has been shown to be effective for photorejuvenation and acne scarring. Previous studies indicated that density, rather than energy, is associated with post-inflammatory hyperpigmentation (PIH) in Asians. The objective of this retrospective study was to assess the efficacy and complications of 'full fraxel' (8 passes) versus 'mini fraxel' (4 passes with same energy and treatment level as 'full fraxel', but double the number of treatment sessions) with the 1550-nm erbium-doped fibre fractional laser (Fraxel SR1500, Reliant Technologies, Inc) in Asian acne scar patients.

Methods: Forty-seven Asian atrophic facial acne scar patients who received full-face 'full fraxel' or 'mini fraxel' treatments between December 2005 and February 2009 were included. All photographic images captured with the Canfield Visia CR system at baseline and follow-up were assessed for clinical efficacy and complications by an independent, non-treating, and blinded physician.

Results: The total densities for 'full fraxel' and 'mini fraxel' were 442.5 MTZ/cm^2 and 210.5 MTZ/cm^2 respectively. For 'full fraxel', the PIH risk was 18.2% with cross-polarised images compared to 6.0% for 'mini fraxel'. This difference was statistically significant ($P<0.001$). Improvement in skin texture, facial acne, acne scarring, enlarged pores, and overall pigmentation irregularity all reached statistical significance at the last follow-up compared to baseline. There was no statistically significant difference in clinical efficacy between three 'full fraxel' and six 'mini fraxel' treatments.

Conclusion: Non-ablative fractional resurfacing is effective and safe in Asians. By reducing the number of passes and total density, the risk of PIH can be reduced. Meanwhile, clinical efficacy can be maintained by increasing the total number of treatment sessions.

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Clinical efficacy of transcutaneous focused ultrasound for non-invasive skin tightening in Asians

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Background: The objective of this study was to determine the clinical efficacy of a focused ultrasound device (Ulthera System) for the treatment of facial skin laxity in Asians.

Methods: The patients received one to three full-face treatments spaced 4 weeks apart with the transcutaneous focused ultrasound device. Two transducers (7.5 MHz, 3.0 mm depth; 4.0 MHz, 4.5 mm depth) were used to deliver a single pass of microthermal areas of coagulation. Standardised photos taken with the Canfield Visia CR system at baseline, 3 months, and 6 months after last treatment were assessed by two independent physicians.

Results: Thirty-one Chinese patients completed a total of 67 treatment sessions. Preliminary objective assessment showed statistically significant improvement for skin laxity along the jawline (P=0.001), cheek (P=0.001), nasolabial fold (P=0.007), mentolabial fold (P=0.028), and infra-orbital fold (P=0.014) at 3 months. Improvement in the jawline (P=0.01), cheek (P=0.016), and mentolabial fold (P=0.041) was maintained at 6 months. Periorbital fine rhytides (P=0.033) also improved at 3 months.

Conclusion: Transcutaneous high-intensity focused ultrasound was effective for facial skin laxity in Asians. Improvement in periorbital fine rhytides was observed. Further studies to optimise treatment parameters may enhance clinical outcomes.

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Promotion of mouse embryonic stem cell differentiation into cardiomyocytes via electrical stimulation

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Introduction: Stem cells have been advocated as a promising therapeutic material for myocardial repair. Embryonic stem cell (ESC), being pluripotent in nature, can differentiate into cardiomyocytes (CMs) in appropriate conditions. Electro-stimulation, previously shown as a trophic factor to elicit phenotypic changes in myoblasts, may also help ESC differentiation into CMs. We aimed to examine whether electro-stimulation could promote CMs differentiation.

Methods: In the present study, D3 mouse ESCs in the form of embryoid bodies (EBs) were first seeded on 0.1% gelatin-coated glass cover slips. Once the EBs had settled down and well attached to the chamber bottom a day after plating, they were subjected to long-term electric field stimulation with an eight-channel C-Pace chronic stimulator (Ion-Optics Co, MA). Voltages of 10 V was used in 1 Hz, 2 ms pulses. Cells were then subjected to functional and molecular data analysis after 7 days of stimulation.

Results and Conclusion: E-stimulation may facilitate CM differentiation as viewed in a number of ways by hyperpolarising RMPs, enhancing cardiac-specific gene expression, aligning cardiac structural protein, and promoting calcium-induced calcium release mechanism. However, further investigation such as its effect on the spontaneous action potentials of beating cells is indeed required to warrant its positive impact on CM differentiation.

Risk factors, clinical features and prognosis of perioperative stroke in adults

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Introduction: Perioperative stroke (POS) is an uncommon but severe surgical complication. No widely accepted guidelines for risk prediction or management have been established. Its prevention depends on knowledge about the nature of this disease.

Methods: A total of 36 cases and equal number of controls in Hong Kong West Cluster hospitals were recruited over 43 months. Peri- and intra-operative features were compared and characteristics of POS were described.

Results: Atrial fibrillation, diabetes mellitus (DM), and history of stroke were identified as risk factors (P=0.017, 0.002, and 0.003, respectively). Prolonged aortic occlusion (P=0.018) and bypass (P=0.002) contributed in cardiac surgery. Only few BP parameters, but not consistently all, were significant; 78% POS were infarcts. Watershed infarction related to hypotension was uncommon. Beta-blocker use seemed to bare protective effect. Blood loss and haemoglobin levels did not correlate to POS. Three-month mortality rate was 36%.

Conclusion: Optimal DM control and cardioversion before elective OT, perioperative anticoagulation in AF and old stroke patients, and beta-blockers may be preventive measures for POS. Role of hypotension in POS aetiology is debatable.

Acknowledgements: This study was supported by Prof KN Lai, Prof DK Luk, Prof ST Fan, Prof YS Ngan (Chiefs-of-Service of Departments of Medicine, Orthopaedics and Traumatology, Surgery, and Obstetrics and Gynaecology respectively, Queen Mary Hospital, Hong Kong).

Short-latency somatosensory-evoked potential in patients with central nervous system space-occupying lesions: a study of 261 cases

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Introduction: Short-latency somatosensory-evoked potential (SSEP) is an electrophysiological technique to study the dorsal column–medial lemniscal sensory system. Its application in central nervous system space-occupying lesions (CNS SOLs) has sparsely been published.

Methods: A total of 261 patients with CNS SOLs underwent SSEP before neurosurgeries. Anatomical locations of the lesions, histopathological diagnoses and prognosis were tried to correlate with the SSEP variables.

Results: The spinal SOLs, especially nerve sheath tumours, was associated with significant abnormalities in various variables including the central conduction time. Other anatomical sites and histopathologies did not correlate with the SSEP findings. Also SSEP did not reflect clinical prognosis.

Conclusion: Short-latency somatosensory-evoked potential is probably not a sensitive test for CNS SOLs except spinal cord lesions. This is probably due to anatomy of the somatosensory pathway. The fact that SSEP has different sensitivities to various tumours may reflect that sensory neurons have heterogenous susceptibilities to different pathologies.

Identification of CD44+ gastric cancer cells by chemotherapeutic enrichment from human gastric cancer

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Introduction: The cancer stem cell hypothesis suggests that tumours are initiated and maintained by a subset of tumourigenic cells capable of self-renewal. Recent studies have demonstrated the existence of cancer-initiating cells in various solid tumours. In this study, we sought to demonstrate the existence of a tumourigenic subpopulation of CD44+ cells within the tumours of gastric cancer patients.

Method: CD44+ and CD44- subpopulations were isolated from gastric tumours of patients and two gastric cancer cell lines AGS and SGC7901 by magnetic bead sorting. Isolated CD44+ and CD44- cells were cultured in undifferentiated conditions to demonstrate their self-renewal ability in vitro. Tumourigenicity of CD44+ cells was demonstrated by subcutaneous injection into SCID mice, and CD44+ cells isolated from established tumour xenografts were re-implanted into secondary mice to study their self-renewal ability in vivo. Dissociated tumour cells from gastric cancer patients were treated with chemotherapeutic agent 5-FU to enrich the CD44+ subpopulation, and the expression and activity of aldehyde dehydrogenase (ALDH) in both CD44+ and CD44- subpopulations were measured by RT-PCR, western blot and flow cytometry. cDNA micro-array analysis of the two subpopulations was also performed to delineate the molecular pathways that contributed to the differential functional effects observed between the two subpopulations.

Results: In-vitro culturing of isolated CD44+, but not CD44- cells, from gastric tumours of patients led to formation of gastric spheroid colonies. These colonies retained CD44+ surface marker expression during culture, and could re-grow into spheroids upon dissociation and re-plating. Subcutaneous injections of as few as 500 CD44+ gastric cancer cells conferred tumourigenicity in SCID mice, and implantation of isolated CD44+ cells from these established tumours remained tumourigenic in successive passages. Upon treatment by 5-FU, CD44+ cells harboured increased ALDH expression as compared with CD44- cells, and cDNA micro-array analysis revealed activation of BMP and Wnt signalling pathways in the CD44+ subpopulation.

Conclusion: Our results demonstrated for the first time the existence of CD44+ cells within the tumours of gastric cancer patients that are endowed with stem cells properties, and also provided a plausible explanation for the chemo-resistance frequently observed in the clinical setting. Such findings provide a basis for further studies on targeting this tumourigenic subpopulation for better eradication of tumour cells in gastric cancer patients.

PIN1 enhances the anti-apoptotic function of Survivin in cancer cells

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Introduction: The peptidyl prolyl *cis/trans* isomerase 1 (PIN1) binds the specific motif comprising a phosphorylated serine or threonine residue preceding a proline (pSer/Thr-Pro) in proteins. PIN1 catalyses isomerisation of the prolyl peptide bond, and subsequently induces conformational changes in the substrates and modulates their functions. Through this mechanism, PIN1 is involved in many cellular events, including cell cycle progression, cell proliferation, and cell transformation. Our previous work has revealed that PIN1 is over-expressed in hepatocellular carcinomas and enhances hepatocarcinogenesis. In this study, we investigated the role of the interaction between PIN1 and Survivin, an inhibitor of apoptosis protein, in hepatocarcinogenesis.

Methods and Results: By co-immunoprecipitation experiments, we found that PIN1 interacted with Survivin via the phosphorylated Thr^{34}-pro motif in Survivin. We then further investigated the effects of PIN1 on the anti-apoptotic function of Survivin using flow cytometry. Through these experiments, over-expression of PIN1 in HeLa and human non-tumourigenic liver MIHA cells were found to suppress the apoptotic response induced by staurosporine through inactivation of caspase-9 and caspase-3. However, the expression of the PIN1 mutants that are catalytically inactive or defective for protein-binding activity did not result in an inhibition of apoptosis. Likewise, the targeted inhibition of PIN1 by small interfering RNA in HeLa and human hepatoma PLC/PRF/5 cells enhanced the apoptotic response induced by staurosporine through caspase-9 and caspase-3 activation. In addition, down-regulation of Survivin by small interfering RNA in PIN1 over-expressing cells abolished the anti-apoptotic effect induced by PIN1, suggesting that the inhibition of apoptosis is mediated through the PIN1-Survivin interaction.

Conclusion: Since phosphorylation of Thr^{34} in Survivin suppresses pro-caspase-9 activation by increasing the binding between Survivin and pro-caspase-9, our results suggest that PIN1 may further regulate the anti-apoptotic role of Survivin through modulation of its binding to pro-caspase-9.

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APPL1 antagonises Tribble 3 in regulating hepatic glucose production through fine-tuning insulin-evoked Akt signalling

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Background: Insulin inhibits hepatic glucose production through activation of Akt signalling cascades. Hepatic insulin resistance contributes to both fasting and fed hyperglycaemia in patients with type 2 diabetes. Our previous study demonstrated that APPL1 is a key player in mediating the glucose-lowering effects of insulin. The major objective of this study was to further characterise how APPL1 modulates insulin-mediated inhibitory effects on glucose production using both ex-vivo experiments and mouse models.

Methods: Primary rat hepatocytes were infected with adenovirus expressing full-length APPL1 or APPL1-specific RNAi or Tribble-3 (TRB3) for 24 hours, followed by starvation for 24 hours, and then treated with insulin (10 nM) for various time-points. Total cell lysate was subjected to co-immunoprecipitation, immunoblotting, real-time PCR analysis. Discontinuous sucrose-gradient ultracentrifugation was employed to separate the mouse liver into cytosolic, plasma membrane and endosomal fractions.

Results: In primary rat hepatocytes, adenovirus-mediated overexpression of TRB3 attenuated insulin-induced phosphorylation of Akt and suppression of the gluconeogenic program, but these effects were reversed by APPL1 overexpression. Western blot analysis revealed that the expression of TRB3 is markedly increased in the liver of db/db diabetic mice. TRB3 caused insulin resistance and diabetes by trapping and inactivating Akt. On the other hand, overexpression of APPL1 counteracted the detrimental effects of TRB3 on suppression of insulin-evoked Akt activation, resulting in improved insulin sensitivity. Subcellular fractionation analysis revealed that both Akt and APPL1, but not TRB3, were translocated from cytosol to the plasma membrane and the endosomes upon insulin stimulation. In addition, insulin-stimulated Akt translocation was significantly enhanced by APPL1 overexpression, but attenuated by APPL1 knockdown.

Conclusions: APPL1 and TRB3 serve as a pair of 'Yin-and-Yang' molecules that tightly control the blood glucose levels through fine-tuning insulin-evoked Akt signalling.

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

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Implication of the obesity-associated genetic variants identified from recent genome-wide association studies in Hong Kong Chinese

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Introduction: Recently, two large-scale genome-wide association (GWA) studies by Thorleifsson et al and Willer et al had identified several novel loci associated with obesity and/or body mass index (BMI). This project aimed to examine these loci for associations with obesity in the Hong Kong Chinese population.

Methods: We investigated 13 genetic loci previously reported to be associated with obesity and/or BMI in a case-control study involving 470 obese cases (BMI ≥27.5) and 700 normal-weight control (18.5≤BMI≤23.0). rs8050136, rs10938397, and rs17782313, which showed most significant associations with obesity in the case-control study, were further studied in the population-based Hong Kong Cardiovascular Risk Factors Prevalence Study (CRISPS) cohort.

Results: Seven single nucleotide polymorphisms (SNPs) showed statistically significant associations with obesity in the case-control cohort ($P_{one\text{-}tailed}<0.05$). These included *GNPDA2* rs10938397; *FTO* rs8050136; *MC4R* rs17782313; *KCTD15* rs29941; *SFRS10-ETV5-DGKG* rs7647305; *SEC16B-RASAL2* rs10913469; and *NEGR1* rs3101336. The combined genetic risk of these seven obesity-associated SNPs was analysed and we observed an increased risk of obesity by 1.36 times for each additional risk allele. In the extension study, rs8050136, rs10938397 and rs17782313 also showed significant associations with BMI.

Conclusion: We have successfully replicated the associations of seven SNPs reported in recent GWA studies with obesity in a Hong Kong Chinese population.

Acknowledgements: This research was supported by a CRCG seeding fund for basic research from the University of Hong Kong to Prof Karen SL Lam.

Inhibition of tumour growth by Raf265 via blockade of Raf/MEK/ERK pathway in colorectal cancer

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Introduction: Deregulation of the Raf/MEK/ERK signalling pathway is commonly observed in colorectal cancer (CRC). Since this signalling pathway plays a central role in controlling cell proliferation, apoptosis and differentiation; therefore, a number of therapeutics targeting on the Raf/MEK/ERK pathway has been established recently. Raf265 is an orally bioavailable small molecule which is a potent inhibitor of wild-type and mutant (eg V600E) B-raf kinases. The study of the effect of Raf265 has entered phase I clinical trial in subjects with locally advanced or metastatic melanoma. However, its effect in CRC has not yet been fully understood. The objective of this study was to examine the functional effects of Raf265 in CRC cells in vitro and in vivo.

Methods: Colorectal cancer cell lines of different B-raf status were used in this study. Cell proliferation upon Raf265 treatment (0-50 μM) was determined using 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay. Cell cycle distribution and cell apoptosis upon Raf265 treatment (0, 1, 5, 10, and 15 μM) were assessed by flow cytometry. Phosphorylation of molecules including MEK and ERK, and eIF4E, and expression of Mcl-1 and cyclin D1 was analysed using western blot. For in-vivo animal studies, subcutaneous tumours were established by subcutaneous injections of $1x10^6$ cells into nude or SCID mice, and tumour growth was monitored. Mice were sacrificed at week 16 or when tumour sizes exceeded 30% of their body weight.

Results: Raf265 was found to significantly inhibit cell proliferation in a dose-dependent manner with IC_{50} at 0.83 to 5.54 μM. Increased annexin V positive cells were observed with escalating dose of Raf265, which is indicative of induction of apoptosis in CRC cells. Dose-dependent increase in G_1 and decrease in S phase population (cell cycle arrest at G_1 phase), and increase in the 'sub-G_0' population was also observed after treatment with Raf265. This was accompanied by the reduction of phosphor-MEK and phosphor-ERK. Down-regulation of Mcl-1 and cyclin D1, which are genes regulating apoptosis and cell proliferation, was also observed. Intraperitoneal injections of Raf265 four times weekly demonstrated significant anti-tumour activity in established tumours of xenograft models. Immunohistochemistry demonstrated a close association between inhibition of tumour growth and inhibition of the extracellular signal-regulated kinases (ERKs) 1/2 phosphorylation in the xenograft tumours, consistent with inhibition of the RAF/MEK/ERK pathway. Additional analyses of microvessel density and microvessel area in the same tumour sections using antimurine CD31 antibodies demonstrated significant inhibition of neovascularisation in xenograft models.

Conclusions: These pre-clinical data demonstrate robust anti-tumour activity of Raf265, providing the basis for exploiting its potential use as a therapeutic for Raf-driven CRC tumours.

Bioavailable testosterone predicts a lower risk of Alzheimer's disease in older men: a 1-year cohort study

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Introduction: There are limited data on the protective effects of testosterone regarding Alzheimer's disease (AD) in older men. The objective of this study was to investigate the protective effects of serum total (TT), bioavailable testosterone (BT), and sex hormone binding globulin (SHBG) levels on the subsequent risk of AD in non-demented Chinese older men.

Methods: This 1-year prospective cohort study was carried out in an ambulatory setting. The subjects were ambulatory community-living non-demented Chinese older men. Morning serum TT, BT and SHBG levels were determined for all subjects at baseline, and 1-year prospective follow-up assessment for dementia and AD were done. Alzheimer's disease was diagnosed by the NINCDS-ADRDA criteria for probable AD and aMCI by the Petersen's criteria.

Results: A total of 153 older men (83% of baseline subjects) completed the 1-year follow-up study. Their mean age was 72.7 years. 6.5% (n=10) developed dementia (converters), all having AD. 93.5% (n=143) did not develop dementia (non-converters). Logistic regression analyses for independent predictors of AD showed that the baseline serum BT level, systolic blood pressure (SBP) and ApoE ε4 genotype were significant independent predictors, after adjustment for age, education, body weight, body mass index, fasting plasma glucose level, serum HDL-C and SHBG levels. The baseline serum BT level was protective against the development of AD, and the adjusted relative risk (RR) of BT was 0.22 (95% CI, 0.07-0.69). Baseline SBP and ApoE ε4 genotype were independent risk factors, with RRs of 1.04 and 5.04 respectively.

Conclusion: Bioavailable testosterone in late life protects against future AD development in older men.

Acknowledgement of funding: SK Yee Medical Foundation of Hong Kong (Project No. 203205), HKU Alzheimer's Disease Research Network of SRT on Healthy Aging

Association between alcohol consumption and cognitive impairment in Southern Chinese elderly persons

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Introduction: Alcohol has diverse effects on cognitive function. The objective of the present study was to investigate the association between alcohol consumption and cognitive impairment in Chinese elderly persons in Hong Kong.

Methods: This was a cross-sectional study of 314 Chinese elderly persons, aged 65 years or over. Socio-demographic, co-morbid diseases, alcohol drinking habits, and Mini-Mental State Examination (MMSE) for cognitive function were obtained by a face-to-face interview. Subjects were categorised into normal cognitive and cognitively impaired groups by education-adjusted MMSE cut-off scores.

Results: The mean (SD) age of the participants was 79.9 (6.5) years. The average weekly alcohol consumption in the cognitively impaired group was significantly higher than that of the normal cognition group (mean [SD], 241.21 g [276.26 g] vs 861.89 g [673.03 g] per week respectively; P<0.001, *t* test). Drinkers with light-to-moderate alcohol consumption were associated with higher MMSE scores than non-drinkers and heavy drinkers. Logistic regression analyses showed that heavy drinkers (>400 g alcohol for men and >280 g for women) were associated with an increased risk of cognitive impairment (OR=4.99; 95% CI, 1.8-13.82), while light drinkers and moderated drinkers (<400 g for men and <280 g for women) were associated with reduced risks (OR=0.32; 95% CI, 0.12-0.86 and OR=0.17; 95% CI, 0.06-0.51, respectively). Exercise and age were independent protective and risk factors respectively.

Conclusion: Heavy alcohol consumption is associated with an increased risk of cognitive impairment while light-to-moderate alcohol consumption is associated with reduced risk among Southern Chinese elderly persons in Hong Kong.

Attitudes towards having an advance directive among Chinese nursing home residents in Hong Kong

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Introduction: Advance directive regarding end-of-life care decisions is very relevant for clinical care of elderly persons in Hong Kong. However, there is a paucity of local data in these issues. The objective of this study was to describe the knowledge and preferences of Hong Kong Chinese elderly nursing home residents regarding having an advance directive.

Methods: A total of 1600 Chinese cognitively normal older nursing home residents underwent face-to-face interviews to explore their attitudes towards having an advance directive.

Results: The mean age was 82.4 years. 33.8% of the subjects were males. 94.2% of the subjects would prefer to be informed of the diagnosis if they had terminal diseases. 77.3% would prefer to stay in their nursing homes till last days of life. 88.0% agreed that it would be good to have an advance directive for them regarding medical treatment in the future. In the final sex- and age-adjusted combined logistic regression model, the independent predictors for having an advance directive included asking for relative advice, wishing to be informed of their terminal illness diagnoses, absence of stroke and having no problems in self-care in EQ-5D.

Conclusions: A high proportion of cognitively normal nursing home residents prefer having an advance directive, which is associated with specific social and clinical factors.

Acknowledgement: Research grants from Health and Food Bureau, SHS-E-08

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Preference and willingness-to-pay for community-based end-of-life care among Chinese old-home residents in Hong Kong: a discrete choice experiment approach

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Introduction: There is no information on the preference and willingness-to-pay for community-based end-of-life care among Chinese old-age home residents in Hong Kong. The objective of this study was to estimate the proportion of old-age home residents who would accept end-of-life care in the community old-age home rather than hospital and the trade-off that they are willing to make between attributes of care.

Methods: A total of 1600 residents of 141 old-age homes were recruited and interviewed face-to-face. Four hypothetical questions were asked to explore preferences for end-of-life care. Using a discrete choice approach, specific questions explored acceptable trade-offs between three attributes: availability of doctors on site, attitude of the old-age home care staff, and additional cost of care per month.

Results: Approximately 30% of respondents prefer end-of-life care in hospital, while 35% of respondents prefer the old-age home and 23% of them would consider it in a better old-age home. A good attitude of staff was the most important attribute of the care site. Respondents were willing to pay an extra cost of HK$38 per month for more coverage of doctor's time and HK$376 for a better attitude of staff in the old-age home. Respondents on government's subsidy (Comprehensive Social Security Allowance Scheme) valued the cost attribute more highly, as expected, validating the hypothesis that those respondents would be less willing to pay an additional cost for end-of-life care.

Conclusions: The findings indicate that approximately one-third of the respondents would accept end-of-life care provided in their old-age homes and further use of community-based end-stage care would be possible if better facilities and services were available. The most important attribute of the old-age home to the residents was the attitude of care staff.

Acknowledgement: Research grants from Health and Food Bureau, SHS-E-08

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A comparison of the performance of the Assessment of SpondyloArthritis international Society (ASAS) classification criteria, European Spondyloarthropathy Study Group (ESSG) classification criteria, and Modified New York (MNY) criteria in a cohort of Chinese spondyloarthritis patients

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Background: The existing Modified New York (MNY) criteria and European Spondyloarthropathy Study Group (ESSG) criteria are defective in early diagnosis of patients with spondyloarthritis. The objective of this study was to reclassify a Chinese cohort of patients with previous expert-diagnosed spondyloarthritis according to the recently issued Assessment of SpondyloArthritis international Society (ASAS) classification criteria for axial spondyloarthritis and the two existing criteria, the ESSG criteria and MNY criteria and to compare the clinical characteristics including disease duration, disease activity, and spinal mobility between patients fulfilling these criteria.

Methods: Consecutive patients diagnosed by expert opinion from a tertiary centre were classified into three groups: the Ankylosing Spondyloarthritis (AS) by MNY criteria; undifferentiated spondyloarthritis (USpA) by ESSG criteria (USpA/ESSG), and those by ASAS classification criteria only (USpA/ASAS). Functional status was studied by Bath Ankylosing Spondylitis Functional Index (BASFI). Disease activity was evaluated by Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and C-reactive protein. Spinal mobility including modified Schober test and chest expansion was determined.

Results: A total of 128 spondyloarthritis patients (92 male and 36 female) were recruited. USpA/ASAS group identified patients with shortest disease duration (9.2±2.3 years, 11.6±3.8, 18.7±2.2 years in USpA/ASAS group, USpA group, and AS group respectively; $P<0.01$). USpA/ASAS and USpA/ESSG groups were better than AS group in terms of BASFI, modified Schober test and chest expansion. C-reactive protein and BASDAI were similar in the three groups.

Conclusion: The ASAS classification criteria are shown to identify spondyloarthritis patients at an earlier stage when spinal mobility and functional status are preserved. This group of USpA patients demonstrated comparable disease activity to other groups, suggesting a need and predictably better outcome for early treatment.

The role of sox genes (group F) in zebrafish primitive haematopoiesis

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Introduction: Sry-related HMG box (SOX) genes belong to a family of transcription factors sharing a conserved high-mobility-group domain. However, information about the functions of group F sox genes (sox 7, 17, 18), particularly in haematopoiesis, is scarce. This is addressed in the present study using morpholino gene knockdown in zebrafish.

Methods: Morpholinos (MO) targeting selected sox genes were injected into zebrafish embryos and were analysed for effects in haematopoiesis using real-time RT-PCR and WISH.

Results: *Sox17* was expressed in neural tube and intermediate cell mass (ICM) while *sox7* and *sox18* were enriched in the vasculature. *Sox32*, unique in teleosti, was expressed predominantly in the posterior ICM. Injection of Sox17 MO resulted in a significant decrease in α embryonic haemoglobin (*alpha-eHB*) expression rescuable by co-injecting *sox17* but not by *sox7* or *sox18* mRNA. Expression of *scl* and *gata-1* was slightly decreased by 22% and 35% respectively. Coinjection of Sox7 and Sox18 MO had no effect on *alpha-eHB* expression. Knockdown of *sox32* resulted in decrease of *alpha-eHB* expression by 42%, while *sox17* expression was unaffected.

Conclusion: Sox17 and sox32 play an important role in the initiation and maintenance of primitive haematopoiesis as shown by decrease of primitive erythroid markers *gata-1* and *alpha-eHB*. Of the four SoxF genes in zebrafish, knockdown of sox17 and sox32 have an effect on primitive erythropoiesis as shown by the decrease in gata-1 and alpha-eHB expression in 18hpf embryos.

Sox7—a potential tumour suppressor in myeloid malignancies

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Introduction: Sry-related HMG box (SOX) genes are a family of 20 proteins characterised by a highly conserved high-mobility-group (HMG) domain. The sox genes have been shown to regulate diverse processes during embryonic development and neoplastic transformation. However, their roles in haematopoiesis and leukaemogenesis are unclear.

Methods: Bone marrow (BM) or peripheral blood samples of patients with myeloid (acute myeloid leukaemia [AML], myelodysplastic syndrome [MDS], chronic myelogenous leukaemia [CML]) and lymphoid (acute lymphoblastic leukaemia [ALL]) malignancies, as well as normal BM and umbilical cord blood (UCB), were prospectively collected. Mononuclear cells (MNC) and CD34+ cells were isolated. Expression of sox genes was evaluated by reverse-transcription polymerase chain reaction (RT-PCR) and western blotting. Methylation of CpG island in sox7 promoter was evaluated by bisulfite sequencing and methylation specific PCR. Leukaemic cell lines were treated with 5-aza-2′deoxycytidine (5-aza-dC).

Results: Sox7 was expressed in normal BM MNC (5/14) and UCB CD34+ (6/6) but not in AML (n=33), CML (n=13) and MDS (n=16), as well as four AML-derived cell lines (ML-2, KG-1, NB4, K562). Sox7 was also expressed in 17/23 ALL cases and a cell line derived from precursor B-cell ALL (Nalm-20). None of the other 19 sox genes showed differential expression between normal, myeloid, and lymphoid malignancies. In silico analysis revealed CpG island within the promoter and exon 1 region of sox7 gene. There was CpG hypermethylation as showed by both bisulfite sequencing and methylation-specific PCR. Treating AML-derived cell lines (KG-1, ML-2, and K562) with 5-aza-dC re-expressed sox7 gene.

Conclusion: Sox7 exhibited differential gene expression between normal haematopoietic cells and myeloid malignancies and was silenced in the latter due to promoter CpG island methylation. Its role as a tumour suppressor gene should be further evaluated.

Distinct roles of MicroRNA-1 and -499 in ventricular specification and maturation of human embryonic stem cells

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Introduction: MicroRNAs (miRs) negatively regulate transcription and are important determinants of normal heart development and heart failure pathogenesis. Despite the significant knowledge gained in mouse studies, their functional roles in human (h) heart remain elusive. We hypothesised that miRs that figure prominently in cardiogenesis are differentially expressed in differentiating, developing, and terminally mature human cardiomyocytes (CMs).

Results: As a first step, we mapped the miR profiles of h embryonic stem cells (ESCs), hESC-derived (hE), foetal (hF) and adult (hA) ventricular (V) CMs. Sixty-three miRs were differentially expressed between hESCs and hE-VCMs. Of these, 29, including the miR-302 and -371/372/373 clusters, were associated with pluripotency and uniquely expressed in hESCs. Of the remaining miRs differentially expressed in hE-VCMs, 23 continued to express highly in hF- and hA-VCMs, with miR-1, -133, and -499 displaying the largest fold differences; others such as miR-let-7a, -let-7b, -26b, -125a, and -143 were also significantly expressed in h fibroblasts, indicating non-cardiac specificity. Functionally, LV-miR-499 transduction of hESC-derived cardiovascular progenitors significantly increased the yield of hE-VCMs (to 72% from 48% of control; $P<0.05$) and contractile protein expression without affecting their electrophysiological properties ($P>0.05$). By contrast, LV-miR-1 transduction did not bias the yield ($P>0.05$) but decreased ADP and hyperpolarised RMP/MDP in hE-VCMs due to increased I_{to}, I_{Ks} and I_{Kr}, and decreased I_f ($P<0.05$) as signs of maturation. Also, LV-miR-1 but not -499 augmented the immature Ca^{2+} transient amplitude and kinetics.

Conclusion: Based on these and additional molecular pathway analyses, we conclude that miR-1 and -499 play differential roles in human cardiogenesis, and their effects are context dependent. While miR-499 promotes ventricular specification of hESCs, miR-1 serves to facilitate electrophysiological maturation.

An inducible transgene expression system for regulated phenotypic modification of human embryonic stem cells

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Self-renewing pluripotent human embryonic stem (hES) cells are capable of regenerating such non-dividing cells as neurons and cardiomyocytes for therapies and can serve as an excellent experimental model for studying early human development. Both the spatial and temporal relationships of gene expression play a crucial role in determining differentiation. To obtain a better understanding of hES cell differentiation, it will be necessary to establish an inducible system in hES cells that enables specific transgene(s) to reversibly and conditionally express (1) at specific levels and (2) at particular time-points during development. Using lentivirus (LV)-mediated gene transfer and a tetracycline-controlled trans-repressor, we first established in hES cells a doxycycline (DOX)-inducible expression system of green fluorescent protein (GFP) to probe its reversibility and kinetics. Upon the addition of DOX, the percentage of GFP(+) hES cells increased time dependently. The time at which 50% of all green cells appeared (T_{50}^{on}) was 119.5±3.2 h; upon DOX removal, GFP expression declined with a half-time (T_{50}^{off}) of 127.7±3.9 h and became completely silenced at day 8. Both the proportion and total mean fluorescence intensity were dose-dependent (EC_{50}=24.5±2.2 ng/mL). The same system when incorporated into murine (m) ES cells similarly exhibited reversible dose-dependent responses with a similar sensitivity (EC_{50}=49.5±8.5 ng/mL), but much faster kinetics (T_{50}^{on}=35.5±5.5 h, T_{50}^{off})=71.5±2.4 hours). DOX-induced expression of the Kir2.1 channels in mES and hES cells led to robust expression of the inwardly rectifying potassium (K^+) current and thereby hyperpolarised the resting membrane potential. We conclude that the LV-inducible system established presents a unique tool for probing differentiation.

Functional characterisation of a novel nucleoporin gene *nup98* in zebrafish embryos

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Introduction: The nucleoporin gene *nup98* is important for the regulation of cytoplasmic-nuclear trafficking. Frequent disruptions of NUP98 during chromosomal translocation in acute myeloid leukaemia suggest that it may play a role in normal haematopoiesis. nup98-knockout mice has resulted in early embryonic lethality. Therefore, its role in embryonic haematopoiesis remains unclear. In this study, we have cloned a zebrafish *nup98* gene and examined its role in embryonic development, with particular reference to haematopoiesis.

Methods: Two expressed sequence tags with translated sequence homologous to human NUP98 were identified. The gene was cloned by PCR from cDNA of zebrafish embryos. Expression of nup98 in zebrafish embryos was investigated spatially by whole-mount in-situ hybridisation and temporally by RT-PCR. The functions of nup98 were examined by morpholino knockdown and the effects on embryonic development evaluated by gene expression studies and confocal microscopy. Cellular functions of zebrafish nup98 were investigated in HeLa cells.

Results: Zebrafish *nup98* gene shared 65% identity to human NUP98 homolog in protein sequence. The gene was expressed during early embryonic development since 1-cell stage and diffusely in eyes and the developing brain since 18 hpf. About 30% nup98-knockdown embryos developed intracranial haemorrhage at 48 hpf, resulting from disrupted blood vessels. nup98-knockdown upregulated pu.1 and scl as evaluated by quantitative RT-PCR. Moreover, ectopic expression of zebrafish nup98 rescued the defective mRNA export due to NUP98 knockdown in HeLa cells.

Conclusion: A novel zebrafish *nup98* gene was shown to exhibit conserved function in mRNA trafficking. Its role in embryonic development should be further evaluated.

Declined frontal white matter integrity in Alzheimer's disease: a diffusion tensor imaging study

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Introduction: Previous studies on structural changes of Alzheimer's disease (AD) have been focused on grey matter atrophy. There is a resurgence of interests on white matter integrity in this prominently increasing patient population. Diffusion tensor imaging (DTI) provides key information on the microstructural changes beyond macroscopic anatomical imaging by in-vivo tracing molecular diffusion in the brain, and the measured fractional anisotrophy (FA) value may represent axonal integrity of neuronal networks. Data of DTI from AD patients are limited, and the literature is controversial regarding whether the AD process has a greater impact on anterior versus posterior cerebral white matter.

Methods: Eighteen patients with mild AD and 16 age-matched healthy adults were recruited into the study. Demographic features of the two groups were comparable. Data of DTI were collected using a Philips 3.0T MRI scanner. Scan parameters were as follows: B_0=800 s/mm^2, FOV=224*224*140 mm, resolution=1.75*1.75*2 mm, non-collinear 15 directions was acquired. 3D T1 anatomy was also collected. We processed DTI data with DTI toolbox, and anatomical T1 data with VBM5 toolbox in SPM. Voxel-by-voxel analysis was applied to compare the difference in FA value, and volume of white matter of the normalised brain between the elderly and AD groups.

Results: Voxel-based analysis showed no significant difference in white matter volume between the two groups, but FA value was reduced greatly in the left anterior cingulate (–10,37,–3), right anterior cingulate (12,0,28), and left medial frontal lobe (–18,32,–12). Minor reduction was found in other brain regions such as body of the corpus callosum, right midbrain (12,–12,–6), right posterior corpus callosum (8,–44,2), and bilateral, especially right temporal lobe (36,–8,–20), upon right hippocampus. Coordinates (x,y,z) were labelled according to Talairach atlas.

Conclusion: DTI could be valid and more sensitive than traditional T1 anatomy in detecting microscopic white matter lesions. Our data showed a greater decrement in FA value over the anterior than posterior brain regions, and this decrement was not due to white matter atrophy. Our findings are in line with the retrogenesis hypothesis which predicts reversed demyelination during the process of AD, as the frontal lobe fibres are myelinated relatively late during brain development. These results also support previous findings of our behavioural study that frontal lobe abnormality might be the neural basis for cognitive deficit in AD patients.

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

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Modification of serum adiponectin and CINC-1 levels by intermittent hypoxia and/or hyperlipidemia in vivo

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Background: Intermittent hypoxia (IH) is a hallmark feature in obstructive sleep apnoea (OSA), which is closely associated with atherosclerosis. Hyperlipidemia is another risk factor of atherogenesis. Adiponectin is an adipokine that exerts anti-inflammatory and anti-atherogenic properties. This study aimed to explore the effects of IH and hyperlipidemia on circulating levels of adiponectin and cytokine-induced neutrophil chemoattractant (CINC)–1 in the rat model in vivo.

Methods: Male S-D rats were randomly divided into four groups: regular chow diet or high-fat high-cholesterol diet (HFHC)–fed (Research Diets, US) plus intermittent normoxia (IN) or IH. The IH exposure was performed using OxyCycler A84 System (BioSpherix, US) daily, which was composed of cycles of 4 min 10% O_2 followed by 2 min 21% O_2 for 6 hours. After 28 days, rats were sacrificed and serum levels of cholesterol, adiponectin, and CINC-1 were measured by ELISA.

Results: The HFHC-fed groups showed an increase of serum total cholesterol levels. The IH or HFHC/IN group caused a decrease in serum adiponectin levels (6.09±0.37 [IH group] or 4.61±0.54 [HFHC/IN group] vs 8.24 ±0.82 μg/mL [IN group], P<0.05 and P<0.01, respectively). The HFHC/IN group caused an increase in serum CINC-1 levels (259.64±40.87 [HFHC/IN group] vs 105.9±20.95 pg/mL [IN group], P<0.05). The IH group also led to upregulation (179.1±12.62 pg/mL, P<0.05) compared to IN group, and to further elevation of serum CINC-1 levels in HFHC/IH group (330.3±21.99 pg/mL, P<0.01).

Conclusion: These data demonstrated that IH led to suppression of serum adiponectin and elevation of serum CINC-1 in our rat model. IH in combination with diet-induced hyperlipidemia synergised the elevation of serum CINC-1, in support of enhanced inflammatory response in subjects with OSA.

Acknowledgement: This research was supported by Hong Kong RGC General Research Fund (HKU 771908M) and HKU-CRCG Seeding Funding for Basic Research.

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In-vitro growth inhibitory effects of arsenic trioxide in non–small-cell lung cancer with different epidermal growth factor receptor mutations

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Introduction: Previous studies have demonstrated differential treatment responses of epidermal growth factor receptor tyrosine kinase inhibitors (EGFR TKIs) in non–small-cell lung cancer (NSCLC) with different EGFR mutation status. In particular, deletions in exon 19 and point mutation in exon 21 (L858R) confer drug sensitivity, while T790M point mutation in exon 20 is commonly associated with resistance. Arsenic trioxide (ATO) has been an established treatment in acute promyelocytic leukaemia in which preliminary data on head and neck cancer suggested its potential regulation of EGFR signallings. Therefore this study aimed at investigating the growth inhibitory effect of ATO treatment in NSCLC with different EGFR mutation status.

Methods: Growth inhibitory effects of ATO and erlotinib (an EGFR TKI) were studied in seven NSCLC cell lines with different EGFR mutation status (wild-type [WT], exon 19 deletion and double mutant [L858R and T790M]) by 3-(4, 5-dimethyl-2-thiazolyl)-2, 5-diphenyl-2H tetrazolium bromide (MTT) assay and Annexin-V/Propidium Iodide assay. Experiments were conducted with varying drug concentrations and time course.

Results: Both ATO and erlotinib work in a time- and dose-dependent manner. When treated with drug concentration of 3 micro molar for 48 h, ATO is more efficient than erlotinib on growth inhibition in the following cell lines NCI-H23 (WT), HCC2935 (del 19), NCI-H1650 (del 19), and NCI-H1975 (L858R/T790M) [mean inhibitory rate ranging from 53.1 to 67.1% for ATO vs –0.5 to 26.53% for erlotinib; P<0.05 for significant difference]. On the other hand, erlotinib demonstrates stronger growth inhibition than ATO in two cell lines with EGFR del 19 (mean inhibitory rate with erlotinib vs ATO in HCC827: 86.7±2.6% vs 45.71±3.0%; in HCC4006: 80.8±2.3% vs 33.31±5.3%; P<0.05). While for NCI-H358 (WT) there is no significant difference between two drugs.

Conclusions: ATO exerts stronger growth inhibition than erlotinib on most of the NSCLC cell lines tested, except two with EGFR exon 19 deletions. However the anti-cancer pathway of ATO seems not relying on EGFR mutation status with an implication on multiple downstream signallings.

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Regulation of cell proliferation by large-conductance calcium-activated potassium and volume-sensitive chloride channels in human cardiac fibroblasts

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Background: Cardiac fibroblasts play a central role in the maintenance of extracellular matrix in the normal heart and as mediators of inflammatory and fibrotic myocardial remodelling in the injured and failing heart. Excessive fibroblast proliferation and increase in the extracellular matrix increase myocardial stiffness and cause ventricular dysfunction and subsequent heart failure. Our previous study demonstrated that multiple ion channels were heterogeneously expressed in human cardiac fibroblasts, including IK_{Ca} (large-conductance calcium-activated potassium current), $I_{Cl.vol}$ (volume-sensitive chloride current), and sodium current (I_{Na}). Little is known about the functional involvement of these ion channels in cardiac fibroblasts, and the present study was therefore designed to examine the possible involvement of these ion channels in proliferation of human cardiac fibroblasts.

Methods and results: Using MTT assay and ^{3}H-thymidine incorporation assay, we found that the I_{Na} current blocker tetrodotoxin had no effect on cell proliferation; however, the specific big conductance I_{KCa} blocker paxilline (1 and 3 μM) and the volume-regulated chloride channel blocker 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid disodium (DIDS, 100-200 μM) remarkably suppressed proliferation of human cardiac fibroblasts with 48 h incubation. Knockdown of KCa1.1 or CLC3 with specific siRNAs significantly reduced I_{KCa} or $I_{Cl.vol}$ channel protein levels, the cell proliferation was decreased by the corresponding siRNA. Flow cytometry analysis showed that human cardiac fibroblasts retained at G_0/G_1 phase (control, 55.8%) by paxilline (3 μM, 79.2%, $P<0.01$) or DIDS (200 μM, 72.8%, $P<0.05$) or the corresponding siRNAs; meanwhile distribution of cells in S phase was decreased. Western blot analysis revealed a reduced expression of the cell cycle regulatory proteins cyclin D1 and cyclin E.

Conclusion: Our results demonstrate that I_{KCa} and $I_{Cl.vol}$ channels, but not I_{Na} channels, participate in the regulation of proliferation of human cardiac fibroblasts by promoting cell cycle progression via modulating cyclin D1 and cyclin E expression.

Acknowledgement: This study was supported by the Research Grant Council of Hong Kong (HKU 760306M).

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Treatment of endothelial progenitor cells dysfunction in patients with type 2 diabetes with human embryonic stem cells–derived endothelial progenitor cells

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Introduction: Clinical studies suggest that endothelial progenitor cells (EPCs) can enhance angiogenesis and improve cardiac function in patients with myocardial ischaemia. However, the use of autologous EPCs therapy in those patients with diabetes is limited by the functional impairment in their EPCs. Recent advance in the availability of EPC derived from human embryonic stem cell (HES) could be a potential cell source to overcome the hurdles of the intrinsic scarcity and phenotypic deficiencies of EPCs.

Methods and Results: We investigated the cytokine profile and angiogenic potential of EPCs isolated from peripheral blood of type 2 diabetic patients (DM-EPC) and healthy controls (C-EPC). Cultured human umbilical vascular endothelial cells (HUVEC) and endothelial cells derived from HES (HES-EPC) were also assessed to evaluate their therapeutic potential. Furthermore, tube formation assay was performed to assess the in-vitro angiogenic potential of different source of EPCs. Using cytokine profiling, two angiogenic factors, vascular-endothelial growth factor (VEGF, 197 vs 664 pg/mL; $P<0.001$) and angiogenin (418 vs 866 pg/mL; $P<0.001$) were shown to be down-regulated in DM-EPC compared with C-EPC. Tube formation assay revealed functional impairment of DM-EPCs which could be rescued by the replenishment of VEGF and angiogenin. More importantly, condition medium from cultured HES-EC, but not from HUVEC could also rescue the functional impairment of DM-EPC ($P<0.05$). When injected into the hind muscles in immunodeficient SCID mice subjected to unilateral ischaemia, $CD34^+CD31^+$ HES-EPC cells but not angiogenic factors cocktail improved limb salvage and haemodynamic ($n=6$, $P<0.05$).

Conclusion: Our results demonstrate that angiogenic factors secreted from HES-EPC can restore the function of EPCs in patients with type 2 diabetes, which can potentially improve the clinical efficacy of EPC therapy in those patients.

Acknowledgements: This study was supported by the CRCG Small Project Funding (Project No. 200807176035 and 200807176179), Outstanding Researcher Award of the University of Hong Kong, and Sun Chieh Yeh Heart Foundation.

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A retrospective comparative analysis of the management of freckles and lentigines using 595-nm long-pulsed dye laser, 755-nm long-pulsed Alexandrite Laser, 532-nm Q-switched Nd:YAG laser, and 532-nm long-pulsed Nd:YAG laser in Oriental patients

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Background: Epidermal pigmentation can be a challenge to treat in Asian patients as the risk of post-inflammatory hyperpigmentation (PIH) is increased. Our objective was to determine the effectiveness and safety of using 595-nm long-pulsed dye laser (LPDL), 755-nm LP Alexandrite laser, 532-nm Q-switched (QS) Nd:YAG laser, and 532-nm LP Nd:YAG laser for management of freckles or lentigines in Chinese.

Methods: Forty Chinese patients with freckles and lentigines were divided into four groups and treated with different lasers. An independent clinician assessed pre- and post-treatment photographs.

Results: There were statistically significant improvements of global and focal facial pigmentation in patients treated with 595-nm LPDL, QS Nd:YAG, and LP Nd:YAG lasers. Optimum improvement was achieved by 50% of patients in the 595-nm LPDL group by 3 months, 60% of patients in QS Nd:YAG group after 3-12 months, and 70% of patients in the LP Nd:YAG group after 6-12 months. Risk of PIH was 38% after 755-nm LP Alexandrite treatment, 33% after QS Nd:YAG, 6% after 595-nm LPDL, and none after LP Nd:YAG treatment.

Conclusion: 595-nm LPDL and 532-nm LP Nd:YAG appear to be more effective with less complications compared to 532-nm QS Nd:YAG and 755-nm LP Alexandrite laser. 595-nm LPDL achieves visible and optimum results in the shortest time, but was associated with a small risk of PIH. 532-nm LP Nd:YAG also achieved excellent long-term results over and is associated with no PIH risk in this study.

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A retrospective comparative analysis of the management of acne post-inflammatory hyperpigmentation using topical treatment, lasers, or combined treatments in Chinese

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Background: Post-inflammatory hyperpigmentation (PIH) can be considered universal pathophysiological response to cutaneous injury in Fitzpatrick type III to VI patients. Our objective was to determine effectiveness and safety of using topical treatment, lasers, or a combination of topical and laser treatments to treat acne PIH in Chinese patients.

Methods: Thirty-four Chinese patients with acne PIH were divided into three groups: treated with topical bleaching creams only, 1064-nm Q-switched Nd:YAG and/or 595-nm long-pulsed dye laser, or a combination of bleaching creams and laser treatment. An independent clinician assessed pre- and post-treatment photographs to determine efficacy and timing to visible and optimum improvement.

Results: There was significant overall improvement of acne PIH in patients treated with topical treatment, and topical and laser treatment. For selected hyperpigmentation, there was significant improvement in all three groups. However, no significant difference was found between the groups. Almost 50% of patients in all three groups had visible improvement as early as 1 month. Optimum improvement was achieved by a majority of patients in all three groups by 3 months. Only one patient developed PIH as a result of laser treatment.

Conclusion: Topical bleaching creams, laser therapy, and a combination of topical and laser treatments appear to be effective for acne PIH in Fitzpatrick type III and IV skin after a month's treatment with little complications. Topical bleaching creams may be considered as first-line therapy for acne PIH, taking into consideration its effectiveness, ease of use, and cost. Combined topical and laser therapy is also effective and may be considered as second-line treatment.

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Transcriptional regulation of UCP4 by nuclear factor κB and its significance in mitochondrial dysfunction

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

Methods: A 2k-bp DNA fragment upstream of the TIS, and a series of 5′-deleted fragments (n=10) of the 5′-flanking region were cloned into a promoterless reporter gene vector, pGL3-basic. The constructs were co-transfected with luciferase expression vector (pRL-TK) into SH-SY5Y and HEK293 cells. Promoter activities were determined as percentages of maximal transcriptional rate of the basic control vector. Putative regulatory elements were identified using the TFSearch software program. Cells were treated with TNFa (NF-κB activator) and were transfected with pIκBαM (NF-κB inhibitor) to study the effects of NF-κB signalling in mediating UCP4.

Results: Minimal promoter activity was observed within 100 bp upstream of the transcription start site (+1). Two putative response elements, Sp-1 and CAAT box, were identified at nucleotide -62/-49 and -33/-27bp, respectively, upstream of the TIS. However, no TATA box was found. Deletion analysis showed that there was a significant increase in transcriptional activity in the presence of both Sp-1 and CATT box; lower but significant promoter activity was observed in the presence of either Sp-1 or CATT box. A NF-κB response element was identified at 515 bp upstream of TIS. Activation of NF-κB by treatments of TNFα increased the UCP4 promoter activity, whereas inhibition by pIκBaM vector resulted in significant suppression. The mRNA level of UCP4 was significantly modulated by NF-κB–mediated stimulation as shown by the quantitative RT-PCR.

Conclusions: We have identified the critical region of human UCP4 promoter consisting of at least two important transcription factors, Sp-1 and CATT box, sufficient to initiate gene transcription. We have also identified an upstream NF-κB binding site, which might be crucial in the transcriptional regulation of UCP4 gene. NF-κB is one of the important regulators of cell survival in response to cellular stress. We have previously shown that UCP4 is protected against MPP^+-induced cell death and oxidative stress. The identification of NF-κB response elements in the promoter region, and its significant modulation of gene expression by NF-κB indicate an important link between the neuroprotective role of UCP4 and oxidative stress.

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Adipocyte fatty acid–binding protein in Kupffer cell as a novel player in the pathogenesis of non-alcoholic fatty liver disease

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Introduction: Obesity is a major risk factor for non-alcoholic fatty liver disease (NAFLD). Our recent study demonstrated that adipocyte fatty acid–binding protein (A-FABP) is an early predictor for the development of obesity-related pathologies, including metabolic syndrome, type II diabetes, and atherosclerosis. An increased level of A-FABP mRNA expression in patients with NAFLD has also been observed. This study aimed to examine the role of A-FABP in the pathogenesis of NAFLD by using different mice models.

Methods: C57 male mice were intra-peritoneally injected with lipopolysaccharide (LPS) to induce acute liver injury or fed with high-fat liquid diet to induce chronic liver injury. Depletion of Kupffer cell was performed by tail-vein injection of gadolinium chloride III to determine the hepatic cell population that expresses A-FABP. Mice were sacrificed after treatment. Serum alanine aminotransferase (ALT) and aspartate transminase (AST) levels were measured by biochemical methods. Quantitative-PCR was performed to determine the expression levels of A-FABP and pro-inflammatory cytokines in liver. Immunohistochemistry and H&E staining were performed to determine the A-FABP distribution, inflammatory status and necrosis in liver.

Results: Hepatic A-FABP mRNA and protein levels were increased following acute injection of LPS or high-fat diet induction. This change was accompanied by elevated liver injury markers, ALT and AST and increased production of pro-inflammatory cytokines. Depletion of Kupffer cells reduced the LPS-induced over-expression of A-FABP in both mouse models, indicating Kupffer cells were the major cell population for A-FABP expression. The pharmacological inhibitor of A-FABP blocked LPS-induced production of pro-inflammatory cytokines in macrophages.

Conclusion: Both endotoxin and high-fat–induced liver injury are associated with elevated A-FABP expression in Kupffer cells, indicating that the role of A-FABP in the pathogenesis of NAFLD.

Acknowledgement: This study was supported by a CRCG grant to R Hoo and RGC grant to K Lam (GRF768209).

Non-cardiac chest pain: natural history and outcome in a prospective 4-year cohort study

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Introduction: Non-cardiac chest pain (NCCP) has not been extensively studied. We follow-up NCCP subjects to determine their natural history, morbidity, mortality, and resource utilisation.

Methods: All patients seen at the Triage Clinic, Department of Medicine, Queen Mary Hospital with chief complaint of chest pain were recruited and followed up for 4 years. Telephone interviews were conducted after clinic evaluation and details of symptoms and medical attention–seeking behaviour noted. Descriptive statistics were used to tabulate results.

Results: A total of 877 (490 females, 387 males; average age, 55 years) patients with chest pain were followed up for 4 years. The most common diagnoses were NCCP (367, 41.8%), functional dyspepsia (219, 24.9%), and cardiac chest pain (132, 15%). Majority of patients only had symptoms occurring once a month. A significant proportion required medical attention or follow-up (up to 73%). Few casualty attendances and hospitalisations were required. Eleven patients died (mean age, 69 years) on follow-up with a mortality rate of 1.3% in this cohort. The most common cause of death was malignancy (8 of 11 deaths, 73%). None of the NCCP patients died on follow-up. In the NCCP cohort, 18.9% and 32.5% of patients continued to experience chest pain at 2 and 4 years respectively. Quality of life was affected in less than 10% of patients on follow-up.

Conclusion: In this hospital-based clinic study, the prevalence of NCCP was 41.8%. Up to a third of the patients still have symptoms 4 years after initial consultation. There was no mortality in this cohort.

Adipocyte fatty acid–binding protein potentiates lipopolysaccharide-induced inflammatory responses through a positive feedback loop involving JNK and activator protein-1 in macrophages

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Introduction: Adipocyte fatty acid–binding protein (A-FABP) is a key player integrating lipid metabolism and inflammation. Expression of A-FABP was induced by several pro-inflammatory stimuli and mice with A-FABP deficiency are protected against several inflammatory diseases. This study investigated the molecular mechanism whereby A-FABP modulates inflammation in macrophages.

Method: Murine macrophage cell line RAW 264.7 cells were stimulated with lipopolysaccharide (LPS) and the mRNA and protein level of A-FABP were quantified by real-time PCR and in-house immunoassay. The transcriptional activity of the promoter was measured by luciferase reporter assay. Streptavidin pulldown assay and chromatin immunoprecipitation were performed to evaluate the association of c-Jun with the promoter. Suppression of c-Jun, A-FABP and c-Jun NH2-terminnal kinase (JNK) were achieved by either siRNA-mediated knocking down or their specific pharmacological inhibitors.

Results: LPS induced A-FABP expression through transcriptional activation. This effect was mediated by JNK, which promoted the recruitment of c-Jun to a highly conserved activator protein-1 (AP-1) binding motif within the proximal region of the A-FABP promoter. LPS-induced A-FABP transactivation was largely abolished by either pharmacological inhibition of JNK, knocking down c-Jun, or mutating the AP-1 recognition site. Vice versa, LPS-evoked phosphorylation of JNK, activation of AP-1 and production of pro-inflammatory cytokines were markedly attenuated by pharmacological or genetic suppression of A-FABP in macrophages. Furthermore, LPS-induced elevation in A-FABP expression was also inhibited by its own selective inhibitor BMS309403.

Conclusion: A-FABP, JNK and AP-1 form a finely tuned positive feedback loop that potentiates LPS-induced inflammatory responses in macrophages. Pharmacological inhibition of A-FABP represents a promising strategy for treating inflammation-related diseases.

Acknowledgements: RGC CRF (HKU 2/07C) and NSFC/RGC (N_HKU 735/08)

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Clarithromycin-amoxicillin–containing triple therapy: a valid empirical first-line treatment for *Helicobacter pylori* eradication in Hong Kong?

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Background: Recent studies have suggested the eradication rate for *Helicobacter pylori* infection with standard amoxicillin-clarithromycin–containing triple therapy as first-line have fallen below 80%. Levofloxacin-containing triple therapy was proposed as an alternative. The aim of this study was to compare the efficacy and tolerability of the standard 7-day clarithromycin-containing triple therapy against the 7-day levofloxacin-containing triple therapy as empirical first-line treatment for *H pylori* infection in Hong Kong.

Methods: Three hundred consecutive *H pylori*–positive patients were randomised to receive either 1-week of EAL (esomeprazole 20 mg twice a day, amoxicillin 1 g twice a day, and levofloxacin 500 mg daily) or EAC (esomeprazole 20 mg twice a day, amoxicillin 1 g twice a day, and clarithromycin 500 mg twice a day). *Helicobacter pylori* status was rechecked by ^{13}C urea breath test 6 weeks after treatment. Patients who failed either of the first-line eradication therapy were invited to undergo *H pylori* susceptibility testing.

Results: *Helicobacter pylori* eradication was achieved in 128 (85.3%) of 150 patients in EAL and 139 (92.7%) of 150 patients in EAC groups, respectively (P=0.043), for both intention-to-treat and per-protocol analysis. More patients in the clarithromycin- than the levofloxacin-containing therapy group developed side-effects from the medication (21.3% vs 13.3%, P=0.060). Nine patients (six from the EAL group and three from the EAC group) who failed their corresponding eradication therapy returned for susceptibility testing. All nine isolates were highly resistant to levofloxacin (MIC >32 μg/mL), while only two of the six isolates from the EAL group were resistant to clarithromycin (MIC >0.5 μg/mL).

Conclusions: The standard 7-day clarithromycin-amoxicillin–containing triple therapy is still valid as the most effective empirical first-line eradication therapy for *H pylori* infection in Hong Kong, as prevalence of primary resistance of *H pylori* to amoxicillin and clarithromycin remains low. Patients who failed their empirical first-line eradication therapy should undergo *H pylori* susceptibility testing to guide further treatment.

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Electrophysiological properties of human-induced pluripotent stem cells

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Human embryonic stem cells (hESCs) can self-renew while maintaining their pluripotency. Direct reprogramming of adult somatic cells to induced pluripotent stem cells (iPSCs) has been reported. Although human ESCs and iPSCs have been shown to share a number of similarities, such basic properties as electrophysiology of iPSCs have not been explored. Previously, we have reported that several specialised ion channels are functionally expressed in hESCs. Using transcriptomic analyses as a guide, we observed TEA-sensitive (IC_{50}=3.3±2.7 mM) delayed rectifier K^+ currents (I_{KDR}) in 105 of 110 single iPSCs (15.4±0.9 pF). I_{KDR} in iPSCs displayed a current density of 7.6±3.8 pA/pF at +40 mV. The activation $V_{1/2}$ was -7.9±2.0 mV, k=9.1±1.5. However, I_{KCa}, I_f, Na_V and Ca_V currents could not be measured. TEA inhibited iPSC proliferation (EC_{50}=7.8±1.2 mM) and viability (EC_{50}=5.5±1.0 mM). By contrast, 4-AP inhibited viability (EC_{50}=4.5±0.5 mM) but had less effect on proliferation (EC_{50}=0.9±0.5 mM). TEA and 4-AP had no effect on iPSC differentiation as gauged by the ability to form embryoid bodies and the expression of germ layer markers after induction of differentiation. Neither IBTX nor apamin had any function effects, consistent with the lack of I_{KCa} in iPSCs. Our results reveal further differences and similarities between human iPSCs and ESCs. A better understanding of the basic biology of iPSCs may facilitate their ultimate clinical applications.

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Angiographic results of everolimus-eluting stents in the treatment of extra-long coronary stenoses (AEETES) study

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Background: In the bare-metal stent era, restenosis after coronary stent implantation is closely related to the lesion length, and hence total stent length is used. This study sought to examine the clinical and angiographic outcomes of a second-generation everolimus-eluting stent (Xience-V or Xience-Prime) in the treatment of extra-long coronary stenoses.

Methods: From March 2007 to October 2009, 54 consecutive and symptomatic patients who had very long coronary stenoses and implantation of stents of a total length of longer than 60 mm were enrolled. Dual antiplatelet therapy was prescribed for 12 months. Restudy angiogram was scheduled at 6 to 9 months. Clinical outcomes were reported during hospitalisation and at 1 year.

Results: The mean age of patients was 69±10 years with male predominance (65%). Diabetes mellitus was found in 25 (46%) patients; the left ventricular ejection fraction was 52±13%; glycoprotein IIb/IIIa inhibitors were used in six (11%) patients only. Each patient received 3.3±0.6 stents; the stent size was 2.7±0.3 mm, and a total stent length of 84±16 mm. Restudy angiogram was performed on 33 patients at 8.6±1.9 months. Restenosis was found in eight (24%) patients; the late loss was 0.40 mm. The use of intravascular ultrasound to guide post-deployment stent size optimisation has been found to be associated with a lower restenosis (6.3% vs 41.2%, $P<0.01$). The in-stent restenosis pattern was focal in six (75%), diffuse in one (12.5%), and complete occlusion in one (12.5%) patients. Two (3.7%) patients developed post-procedure myocardial infarction. A total of 30 (55.6%) patients have reached 1-year follow-up. There was one death, no myocardial infarction, and seven target vessel revascularisations, leading to a major adverse cardiac event rate of 26.7%.

Conclusions: The use of everolimus-eluting stents to treat extra-long coronary stenoses is safe. However, the long-term prognosis is hampered by a relatively high restenosis and revascularisation rate, which could be improved by the use of intravascular ultrasound to guide the post-deployment stent size optimisation.

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MicroRNA and cell cycle of embryonic stem and induced pluripotent stem cells: insights for eliminating tumourgenicity

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Purpose: Human embryonic stem cells (hESCs) can self-renew while maintaining their pluripotency; direct reprogramming of adult somatic cells to induced pluripotent stem cells (iPSCs) enables the generation of patient-specific cells for autologous transplantation. MicroRNAs (MiRs) are non-encoding RNAs that function as negative transcriptional regulators via degradation or inhibition by RNA interference. Given that tumourgenicity primarily arises from pluripotent cells, a better understanding of their poorly defined cell cycle properties and regulation by miRs may lead to novel strategies for arresting undesirable cell division of tumourigenic cells.

Method: A combination of lentivirus (LV)-mediated somatic gene transfer, high-resolution live-cell imaging, 'snap-shot' cell cycle analysis (via flow cytometry) and bioinformatics was employed. In brief, the chromosomes of ES/iPSCs were genetically labelled with the histone (H2B)-GFP fusion protein for tracking highly dynamic mitotic (M) events in ES/iPSCs. For characterising the complete cell cycle (G1/S/G2/M), BrdU was incorporated for assaying changes in the DNA content along with the use of cell cycle–specific makers. MiR profiling was performed using microarrays. Results obtained under control and various experimental conditions (eg pharmacological and LV-miR interventions) were compared and contrasted.

Results: Our data revealed that that ES/iPSCs were similarly and highly proliferative with >70% in S and G2/M phases (n=3 for each). The mitotic events in ESCs went to completion within 60 min (n=20). K^+ channel blockade in hiPSCs significantly inhibited their proliferation, primarily by arresting the mitotic phase. A number of miRs with potential roles in pluripotency (eg the miR-302 and -371/372/373 clusters) and cell cycle properties (eg miR-21) as well as their gene targets have been identified.

Conclusion: ES/iPSCs cells were highly proliferative which could be attributed to a dramatically shortened G1 phase and transition to the S phase. K^+ channel blockers inhibited proliferation by arresting mitosis. Bioinformatic and profiling analyses revealed a number of novel miR candidates of potential importance in pluripotency and cell cycles. Further experiments are currently underway.

What can we learn from JUPITER?

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Introduction: The randomised, double-blind, placebo-controlled JUPITER trial reported outcomes in 17 802 subjects (median age, 66 years) with C-reactive protein concentrations ≥2 mg/L and no other coronary heart disease risk factors, followed up for a median of 1.9 years. The study was prematurely terminated due to a 'favourable' impact on the primary (composite) endpoint, without due consideration of absolute effects. We therefore set out to derive number needed to treat (NNT)/year values to gauge absolute benefit or harm.

Methods: Using data reported in JUPITER, we calculated crude values for relative risk (RR) and NNT/year together with 95% confidence intervals (CIs). Data from the 4S study was used for comparison.

Results: Calculated values (95% CIs) for these parameters are shown in the table*

Trial	Subjects†	Event†	%RR (range)	NNT/year (range)
4S	Cholesterol ↑ and CHD	CHD death and non-fatal MI	69 (61 to 79)	63 (49 to 89)
JUPITER	Cholesterol not ↑ No CHD CRP ↑	Primary composite end-point‡	57 (46 to 70)	155 (144 to 242)
		Fatal and non-fatal MI	46 (30 to 70)	457 (298 to 985)
		Death from any cause	80 (66 to 97)	317 (179 to 1403)
		Physician reported DM	125 (104 to 150)	–313 (–173 to –1607)§

* See Kumana CR et al 2009, Evidence Based Medicine 14:70 for all references cited
† CHD denotes coronary heart disease, MI myocardial infarction, and DM diabetes mellitus
‡ Non-fatal MI, stroke, hospitalisation, revascularisation procedure, or cardiovascular death
§ Negative NNT = number needed to harm

Conclusion: Small absolute benefits are off-set by a small absolute risk of acquiring diabetes mellitus.

Herpes simplex encephalitis: how good are we in diagnosing this condition?

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Introduction: Herpes simplex encephalitis (HSE) is the commonest sporadic infective encephalitis in Hong Kong. Early recognition of HSE, which relies on a high index of suspicion, is important as effective treatment is available. Empirical acyclovir is advocated for all cases of clinically suspected viral encephalitis. Electroencephalography (EEG) is a routine investigation in suspected HSE.

Methods: The EEG database of Neurodiagnostic Unit, Queen Mary Hospital, was reviewed retrospectively. All referrals from April 2006 to March 2009 with a diagnosis of suspected HSE treated with empirical intravenous acyclovir were identified. Their presenting features, imaging and laboratory findings, and final diagnoses were reviewed.

Results: During the study period, 60 patients (mean age, 51 years; range, 18-90 years, M:F ratio=13:7) underwent EEG for suspected HSE. Presenting features included fever (n=39), confusion (n=39), impaired consciousness (n=31), focal signs (n=15, seizure in 8), and headache (n=13). All patients underwent brain CT and 45 had MRI. The commonest imaging findings were unrelated old changes (n=20) and normal study (n=16). Lobar inflammation was detected in four patients. EEG was normal, showed diffused abnormalities, or focal/multifocal abnormalities in 16, 31, or 13 patients, respectively. Lumbar puncture was performed in 59 patients. Total cell count was ≤10×10^6/L in 68% of patients and CSF protein was <0.8 g/L in 51% of patients. Polymerase chain reaction for herpes simplex virus was positive in one out of 56 requests. Viral encephalitis was the final diagnosis in three patients (HSE=1, Japanese encephalitis=1, other virus=1). Other common diagnoses included meningitis (n=9), non-CNS sepsis (n=9), psychiatric illnesses (n=8), epileptic seizure (n=6), and acute stroke (n=5).

Conclusion: Our findings demonstrate that we were exercising a high index of suspicion for diagnosing HSE. Our liberal use of empirical acyclovir was also consistent with the IDSA (Infection Diseases Society of America) recommendations. Despite our low threshold of investigating for HSE, only one case was identified over 3 years, suggesting HSE is an uncommon condition.

Ischaemic stroke related to branch artery disease: a missing link?

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Introduction: Clinicians and researchers often classify atherosclerotic cerebral infarctions into large artery atherothrombotic disease (LAD) and small artery lacunar infarction (LACI), but this system of 'dichotomisation' cannot account for a substantial proportion of stroke cases. Twenty years ago, a third mechanism for cerebral infarction—branch artery disease (BAD)—was proposed. However, this concept was understudied and still remains an obscure entity.

Methods: Stroke patients admitted under the Neurology Unit of Queen Mary Hospital over a 24-month period were studied retrospectively. Patients with ischaemic stroke presumably due to atherosclerotic disease were classified according to their imaging +/– clinical findings into three groups: LAD, BAD, and LACI. Patients with BAD were further categorised into five BAD stroke syndromes based on radiological criteria. Clinical characteristics, vascular risk factors, results of vascular workup, and outcome among the various stroke subgroups were compared.

Results: A total of 720 patients with a diagnosis of stroke were admitted during the study period, including 123 LAD (17% of all stroke patients or 33% of all studied patients), 147 BAD (20% or 40%), and 102 LACI (14% or 27%). Among the BAD patients, the number of cases involving Heubner's artery, lenticulostriatal arteries, anterior choroidal artery, thalamoperforating/geniculate arteries or paramedian pontine infarction were 0, 47, 45, 15 or 40 (0, 32, 31, 10 or 27%), respectively. Patients with BAD were the youngest among the three groups. As compared to LAD patients, BAD patients had lower NIHSS scores, were less often diabetic, and carotid stenosis was less common, while stenosis of the intracranial arteries were more frequently seen in BAD as compared to LACI patients. Mean follow-up period was 1085 days, and outcome of BAD patients was intermediate between LAD and LACI. Comparison of variables among the BAD stroke syndromes showed that they were a homogenous group of conditions.

Conclusion: Despite being a rarely applied concept, BAD is the most prevalent subtype of ischaemic stroke in our study. The homogeneity among the BAD syndromes suggests they might represent a distinctive stroke entity. Although patients with BAD and LACI had similar degrees of neurological deficits on presentation, outcome in the former group was significantly worse than the latter.

Generation of human-induced pluripotent stem cells in feeder-independent, serum-free culture system with defined factors

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Introduction: Induced pluripotent stem cells generated from human adult somatic cells through reprogramming hold great promises for future regenerative medicine. However, exposure of human-induced pluripotent stem cells to animal feeder cells and serum in the process of their generation and maintenance imposes risk of transmitting animal pathogens to human subjects, thus hindering the potential therapeutic applications. Our aim was to generate patient-specific human-induced pluripotent stem cells in a feeder-independent culture system with defined factors.

Methods: Fresh human dermal fibroblasts obtained from two healthy volunteers were reprogrammed with a defined set of transcription factors using lentiviral vectors in a feeder-independent cell culture system with defined culture medium.

Results: Two new human-induced pluripotent stem cell lines were generated from dermal fibroblasts of the two subjects under feeder-independent culture system with defined factors. The resultant cells maintained normal karyotypes and expressed a panel of pluripotency markers including stage-specific embryonic antigen (SSEA)–4, tumour-rejection antigen (TRA)-1-60 and TRA-1-81, and alkaline phosphatase. In addition, these cells can be induced to differentiate along lineages representative of the three embryonic germ layers upon formation of embryoid bodies, indicating their pluripotency. Furthermore, subcutaneous transplantation of these cells into immunodeficient mice resulted in teratoma formation.

Conclusion: Our findings are an important step towards generating patient-specific human-induced pluripotent stem cells in a more clinically compliant manner by eliminating the needs of animal feeder cells.

Differentiation of endothelial progenitor cells from human-induced pluripotent stem cells

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Introduction: Experimental and clinical studies have shown that endothelial progenitor cells (EPCs) can enhance angiogenesis in ischaemic myocardium. However, the use of autologous EPCs transplantation isolated from patients is limited by their number and proliferative potential. Human-induced pluripotent stem cell (hiPS)–derived EPC is a potential alternative cell source due to their autology, high power of proliferation and pluripotency.

Methods: We characterise the phenotype and function of hiPS-derived EPCs and compare them with human endothelial cell line (HUVEC) and human embryonic stem cell–derived EPC (H1-EPC). Embryoid bodies (EB) were obtained from hiPS (KS1) and culture for 7 days. Then the EB were plated and cultured in EGM medium for endothelial differentiation. After 2 weeks of differentiation, CD31+/CD34+ cells were sorted by MoFlo-XPD Cell Sorter and subsequently cultured for analysis. Cells were collected weekly for fluorescence staining of EPC markers of Di-acetyl-LDL-DiI and lectin, and flow cytometry analysis for their expression of surface markers endothelial cell (von Willebrand factor [vWF]). Furthermore, their angiogenic property was verified by tube formation assay using HUVEC line as intrinsic endothelial cell control.

Results: Positive stain of Di-acetyl-LDL-DiI and lectin were observed in both iPS-EPC and H1-EPC. Positive stain of vWF and alpha smooth muscle actin further confirmed the EPC phenotype. The numbers of iPS-derived vWF+ve cells progressively increased during 3 weeks of the differentiation after CD31 and CD34 selection (from 21.3% to 32.6%). After 3 weeks of culture, the number of CD34+ve cells (25.9% vs 20%, P>0.05) and vWF+ve cells (69.1% vs 68%, P>0.05) observed were similar between hESC-derived EPC and HUVEC. Furthermore, tube formation assay revealed similar potential in forming capillary with hiPS-derived EPC as H1-EPC and HUVEC (15108±984.6 vs 14867.12±934.54 and 15349.59±1034.67 AU/well).

Conclusions: Our results demonstrate that hiPS-derived EPC resemble normal human endothelial cells with similar phenotypes and angiogenic function but unlimited proliferation capacity. These findings suggest that hiPS-derived EPC can be used as potential alternative cell source for treatment of ischaemic myocardium.

Which chronic disease affects quality of life the most?

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Introduction: The quality of life is as important as the years of life. Chronic diseases may not kill but can impair quality of life. This study aimed to quantify and rank the severity of different chronic diseases by their negative quality of life impact. The SF-6D is a preference measure of health that can give a summary index on quality of life with a range from 0 (dead) to 1 (perfect health).

Methods: A Hong Kong population–specific SF-6D scores of 2410 Chinese adults randomly selected from the general population in Hong Kong were calculated. The SF-6D scores of different chronic disease groups were compared with those of people without any chronic disease by independent *t* tests. Multivariate forward stepwise linear regressions were used to determine the effects of the total number of chronic diseases and individual chronic conditions on the SF-6D scores, controlling for sociodemographic factors.

Results: 38% had chronic diseases and 37% of whom had more than one disease. Population mean SF-6D score was 0.79. The SF-6D score of people with chronic diseases were significantly lower, ranging from 0.68 (mental and pulmonary) to 0.76 (hypertension). There was an inverse linear relationship between the total number of chronic diseases and the SF-6D score with a reduction of 0.06 in preference value for every chronic condition.

Conclusions: The total number of chronic disease has a greater impact on quality of life than individual chronic diseases. Mental illness and chronic lung disease discounted quality of life more than chronic heart diseases, which deserve more health resources.

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Brief problem-solving therapy in primary care had short-term benefit for elderly screened positive of psychological problems

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Introduction: Unrecognised psychological problems are common in the elderly. This study aimed to find out whether screening followed by brief problem-solving therapy by primary care doctors (PST-PC) could improve health-related quality of life (HRQOL) and reduce consultation rates for the elderly.

Methods: A single-blind randomised placebo controlled trial in two GOPC. A total of 299 Chinese patients aged 60 screened positive of psychological problems by the Hospital Anxiety & Depression Scale were randomly allocated to PST-PC (n=149) or video-viewing (placebo) [n=150] groups. SF-36 scores and consultation rates were measured at baseline, 6, 12, 26, and 52 weeks.

Results: Study completion rates were 69 to 70% for the two groups. The SF-36 role-emotional and mental component summary scores improved at week 6 in the PST-PC group but not in the placebo group. Several SF-36 scores improved significantly in the placebo (video) group at weeks 6 to 52. Mixed effects analysis adjusting for baseline values and confounders did not show any difference in any of the outcomes between the PST-PC and placebo (video) groups. There was no change in consultation rates in both groups.

Conclusions: Screening followed by brief PST-PC was associated with a short-term improvement in HRQOL in Chinese elderly patients screened positive of psychological problems. The health benefit of group activities should be further explored.

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Gene expression profiling in lung adenocarcinomas reflects possible different molecular pathogenesis with respect to gender and smoking status

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Introduction: Lung cancer is the leading cause of cancer morbidity and mortality worldwide. There is a prominence of female non-smokers among patients with lung adenocarcinomas in the Asia-Pacific regions, especially among Chinese in Hong Kong. The hypothesis of this study was that discriminatory gene expression could be identified with respect to smoking status in lung adenocarcinomas from male and female patients respectively. The aim of this study was to delineate the expression signatures of lung adenocarcinomas by microarray analysis, with segregation analysis according to gender and their respective smoking status.

Methods: Total RNA from 49 lung adenocarcinomas were processed according to standard protocol from Affymetrix and then hybridised onto Affymetrix GeneChip HG-U133 set with one sample per GeneChip set. Data analysis was done with standard software packages for microarray analysis and validated with an independent microarray (47 lung adenocarcinomas) datasets. Differential gene expression was obtained with log-transformed signal intensities and significance analysis of microarray. Support Vector Machine Learning with leave-one out analysis was used to identify those differentially expressed genes that allows for prediction of adenocarcinomas with respect to different clinical characteristics of gender and smoking status.

Results: Discriminatory gene expression signatures were found in male smokers compared to male non-smokers; and in females, a different list of discriminatory genes between non-smokers and smokers.

Conclusion: There are different discriminatory gene expression signatures in lung adenocarcinomas in relation to gender segregated into different smoking habits. This may give insight into the molecular pathogenesis of lung adenocarcinomas with respect to gender and smoking status.

Endothelial lipase and reverse cholesterol transport in type 2 diabetes mellitus

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Background: Endothelial lipase (EL) is a phospholipase with little triacylglycerol lipase activity and plays an important role in the metabolism of HDL. Modulation of HDL by EL may affect reverse cholesterol transport. We have investigated whether serum EL concentration is associated with changes in the serum capacity to induce cholesterol efflux in patients with type 2 diabetes mellitus.

Methods: A total of 172 diabetic patients and 175 controls were recruited. Serum EL was measured by ELISA and cholesterol efflux to serum was determined by measuring the transfer of [3H]cholesterol from Fu5AH cells to the medium containing the tested serum.

Results: Diabetic patients had significantly higher plasma triglyceride ($P<0.01$), lower HDL cholesterol ($P<0.01$) and elevated high sensitivity C-reactive protein (CRP) ($P<0.01$) compared to the controls. Serum EL was significantly increased in the diabetic patients (27.7±16.6 ng/mL vs 24.0±11.3 respectively, $P<0.05$) and cholesterol efflux to serum was impaired (15.1±2.5% vs 16.7±3.1 respectively, $P<0.01$). In the control subjects, serum EL correlated inversely with cholesterol efflux to serum ($r= –0.16$, $P=0.03$) but no significant association was seen in the diabetic patients. Linear regression shows that in the controls, plasma HDL, serum EL and waist circumference were the major independent determinants of cholesterol efflux to serum whereas in the diabetic cohort, the major independent determinants of cholesterol efflux to serum were HDL, age, and CRP.

Conclusion: Although serum EL concentration was increased in type 2 diabetic patients, impaired serum capacity to induce cholesterol efflux was mainly related to low HDL and subclinical inflammation in these patients.

Effectiveness of ultrasonography screening for renal cell carcinoma in renal transplant recipients

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Introduction: The incidence of malignancy is increased in renal transplant recipients, in which renal cell carcinoma (RCC) is found to be 15 times greater. However, there is still no strong evidence to support regular screening using ultrasonography (USG). In our centre, we arrange USG screening for our renal transplant recipients, particularly for those with acquired cystic disease. Herein, we report the effectiveness of the screening and the outcomes of the sub-clinical RCC.

Methods: All renal transplant recipients in one transplant centre were scheduled to have a USG scanning when they returned for follow-up. The results of screening over a 10-year period were studied.

Results: Of the total 400 transplant patients, six tumours in the native kidneys (five unilateral and one bilateral) were detected in five patients. The average ages at transplantation and diagnosis of RCC were 38.8±6.61 and 48.7±5.76 years, respectively. All tumours were detected by ultrasound scan and then confirmed by computed tomography with contrast. No biopsy was performed before nephrectomy. The patients were neither symptomatic nor polycythemia (haemoglobin, 14.0±1.0 g/dL) and they all subjected to have total nephrectomy. The median of the tumour size was 3.5 (range, 1.5-6.5 cm). The cell types were 4 for papillary and 2 for clear cell, respectively. All the tumours were Furhman grade 1 or 2 with stage 1 of the TNM staging. The patients are alive with stable allograft function and there is no evidence of recurrence on their latest follow-up.

Conclusion: We have shown that USG is an effective screening test to detect early stage of RCC. We recommend renal transplant recipients should have annually USG screening of their native kidneys such that earlier stage of tumour could be detected.

Ketanserin attenuates cigarette-mediated oxidative stress in human bronchial epithelial cells

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Introduction: Chronic obstructive pulmonary disease (COPD) is characterised by progressive airway limitation. Although the pathology of COPD is not fully understood, disruption of the oxidant-antioxidant homeostasis has been suggested to stimulate inflammatory reactions in the lung (Crapo, 2003). Cigarette smoke (CS), one of the major causes of COPD, is abundant in reactive oxygen species (ROS) that can induce oxidative stress. Ketanserin, a selective serotonin receptor 2 (5-HTR_2) antagonist, has been reported to improve lung function in COPD patients (Cazzola et al, 1990) through unknown mechanisms. On the other hand, activation of 5-HTR_2 has been suggested to generate ROS in rat renal mesangial cells (Greene et al, 2000). We therefore hypothesise that 5-HTR_2 is involved in CS-mediated oxidative stress, leading to inflammatory responses in the lungs. The present study aimed at investigating whether ketanserin protects bronchial epithelial cells from CS-induced oxidative stress and inflammatory responses.

Methods: The human bronchial epithelial cell line (BEAS-2B) was cultured to 80% confluence in complete keratinocyte-SFM before treatment. Release of a pro-inflammatory marker, IL-8, was determined by ELISA. Oxidative stress was assessed by the ratio of reduced glutathione (GSH) to glutathione disulfide (GSSG) using spectrophotometric assay.

Results: Our results demonstrated that CS significantly increased the IL-8 level (23.1±4.5 vs 8.5±0.9 pg/mL for CS-exposed and control cells respectively; $P<0.01$) and decreased the GSH/GSSG ratio (21.2±4.6 vs 43.9±10.5; $P<0.001$). Ketanserin (10 nM) completely reversed CS-induced IL-8 elevation and significantly restored CS-induced oxidative stress.

Conclusion: These data indicate the possibility that ketanserin may be beneficial in restoring CS-mediated oxidative stress and hence inflammatory responses.

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

Triiodothyronine promotes cardiac differentiation of embryonic stem cells through PI3K-Akt pathway

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Introduction: Thyroid hormones (3,5,3′-triiodothyronine [T_3]) play a crucial role in cardiac physiology and are implicated in the development of cardiac hypertrophy. Thyroid gland and heart have close temporal and spatial relationship during embryonic development. The effects of T_3 through cardiac foetal gene programming on adult cardiomyocytes have been well characterised; however, it is unclear whether T_3 exerts any effects on cardiac differentiation of embryonic stem cells (ESCs).

Methods: Murine embryonic stem cell (mESC), D3, was used for cardiac differentiation in the current study. Troponin-T positive cells were counted as the ESC-derived cardiomyocytes (ESC-CM) by FACS. RT-PCR analysis revealed the mRNA expression of cardiac specific markers and calcium handling proteins. Furthermore, the calcium handling properties of ESC-CMs were examined by fluorescence confocal microscopy. The underlying mechanism of cardiac differentiation mediated by PI3-Akt pathway was studied by western blotting.

Results: T_3 promotes cardiac differentiation of ESCs as evidenced by an increase in the number of troponin-T positive cells counts. Consistently, mRNA levels of early and late cardiac markers including NK2 transcription factor–related locus 5 (Nkx 2.5), myosin light chain 2 ventricular transcripts (MLC2V), alpha- and beta-myosin heavy chain (A and B-MHC) are also increased with T_3 treatment. Functionally, ESC-CMs treated with T_3 appear to have a more mature calcium handling phenotype with a significant increase in the maximum upstroke velocity, which agreed with the up-regulated ryanodine receptor-2 (RyR2) level. Furthermore, inhibition of PI3/Akt signalling by LY294002 hence suggesting T_3 induces cardiac differentiation and maturation via the Akt pathway.

Differential NOD/SCID mouse engraftment of peripheral blood CD34$^+$ cells isolated from patients with polycythemia vera and secondary polycythemia

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Background: A gain-of-function *Jak2*^{V617F} mutation commonly occurs in polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). We tested if it could account entirely for the neoplastic proliferation of PV by evaluating the NOD/SCID repopulating potential of CD34$^+$ cells from PV and secondary polycythemia patients (SP) and the *Jak2*^{V617F} clone size before and after transplantation.

Methods: CD34$^+$ cells were isolated from all peripheral blood (PB) and some bone marrow mononuclear cells (BMMNC) samples. The cells were transplanted intra-femorally or intravenously into NOD/SCID mice. Engraftment was based on the presence of human CD45$^+$ mouse 45.1$^-$ cells in the recipient BM 6 to 8 weeks after transplantation. Quantification of the Jak2^{V617F} clone was evaluated by real-time quantitative PCR and ARMS-PCR.

Results: Seven of the 26 mice transplanted with 4/13 PBCD34$^+$ cells from PV patients (median, 4.26%; range, 0.3-5.56%), in contrast to 0/14 mice from nine SP patients engrafted (P=0.0168). The engrafting PV cells were of multi-lineage. Jak2^{V617F}/Jak2^{Total} ratios decreased after transplantation, especially when the uninjected BM was analysed (before: 65.9% vs after: 13.0%; P=0.001). When BMMNC were transplanted, 8/9 mice from 4/4 PV, 4/5 mice from 2/3 ET but 0/7 mice from five PMF patients engrafted. In PV and ET BMMNC transplants, the Jak2^{V617F} clone significantly diminished but in PMF it was more sustained.

Conclusion: The superior engrafting potential of PB CD34$^+$ cells in PV patients and the reduction of Jak2^{V617F} clone upon engraftment suggested the proposition that events other than Jak2^{V617F} mutation might play a role in the pathogenesis of PV.

A novel zebrafish jak2a^{V581F} model shared features of human JAK2^{V617F} polycythemia vera

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Objective: The Janus kinase 2 (JAK2) is important for embryonic primitive haematopoiesis. A gain-of-function *JAK2* (*JAK2*^{V617F}) mutation in human is pathogenetically linked to polycythemia vera (PV). In this study, we generated a zebrafish orthologue of human *JAK2*^{V617F} (referred herewith *jak2a*^{V581F}) by site-directed mutagenesis and examined its relevance as a model of human PV.

Methods: Zebrafish embryos at 1-cell stage were injected with *jak2a*^{V581F} mRNA (200 pg/embryos). In some experiments, the embryos were treated with a specific JAK2 inhibitor TG101209. The effects of jak2a stimulation on haematopoiesis, jak/stat signalling and erythropoietin signalling were evaluated at 18 hours post-fertilisation (hpf).

Results: Injection with *jak2a*^{V581F} mRNA significantly increased erythropoiesis, as enumerated by flow cytometry based on gata1$^+$ population in dissociated Tg(*gata1:gfp*) embryos. The response was reduced by *stat5.1* morpholino co-injection (control: 4.37±0.08%; *jak2a*^{V581F} injected: 5.71±0.07%, co-injecting *jak2a*^{V581F} and *stat5.1* morpholino: 4.66±0.13%, P<0.01). *jak2a*^{V581F} mRNA also up-regulated *gata1* (1.83±0.08 fold, P=0.005), *embryonic* α (α*eHb*: 1.61±0.12 fold, P=0.049) and β*-haemoglobin* gene expression (β*eHb*: 1.65±0.13 fold, P=0.026) and increased stat5 phosphorylation. These responses were also ameliorated by *stat5.1* morpholino co-injection or treatment with a specific JAK2 inhibitor TG101209. *jak2a*^{V581F} mRNA significantly reduced *erythropoietin* gene (0.24 ±0.03 fold, P=0.006) and protein expression (control: 0.633±0.11; *jak2a*^{V581F} mRNA: 0.222±0.07 mIU/mL, P=0.019).

Conclusion: The zebrafish *jak2a*^{V581F} model shared many features with human PV and might provide us with mechanistic insights of this disease.

Successful engraftment by leukaemia-initiating cells in adult acute lymphoblastic leukaemia after direct intra-hepatic injection into unconditioned newborn NOD/SCID mice

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Objective: Xenogeneic transplantation has been the gold standard for the enumeration of leukaemia-initiating cells in acute myeloid leukaemia and acute lymphoblastic leukaemia (ALL). Most transplantation models have required conditioning in which the recipients were either irradiated or treated with chemotherapy prior to injection of human leukaemia cells. In this study, we reported a hitherto undescribed model in which adult ALL cells were injected into unconditioned newborn NOD/SCID mice via an intrahepatic route.

Methods: Bone marrow (BM) and peripheral blood (PB) were collected from patients with ALL at diagnosis or relapse. $CD34^+$ selected lymphoblasts or mononuclear cells were transplanted as aforementioned. The cells were also transplanted into sublethally irradiated adult mice via intravenous route for comparison. Leukaemia engraftment was enumerated from mouse BM 6 to 18 weeks after transplantation. Clonality of the engrafting cells was examined based on *IGH* rearrangement and fluorescence in-situ hybridisation.

Results. Five out of 13 ALL samples engrafted into the recipient BM 6 to 18 weeks after transplantation. The engrafted cells recapitulated the immunophenotype and cytogenetic characteristics of the original samples. Engraftment in BM and PB was significantly correlated. Importantly, there was significant correlation of engraftment between this and the conventional adult NOD/SCID mouse model involving irradiation.

Conclusion. Our results demonstrated that this unconditioned newborn mouse model could be used for the enumeration of LIC in ALL and should be further evaluated.

Cognitive predictors of rehabilitation outcomes of stroke patients in Hong Kong

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Introduction: Stroke is a leading cause of disability in adults. This study aimed to identify cognitive predictors of rehabilitation outcomes of stroke patients.

Methods: Forty-four patients who had first-time stroke were assessed with neuropsychological tests, Geriatric Depression Scale (GDS), and Functional Independence Measure (FIM) when they were discharged from an in-patient rehabilitation hospital (Time 1). They were reassessed 3 months later (Time 2). The relationship between the cognitive functioning at Time 1 and the rehabilitation outcome at Time 2 was examined via univariate correlational and linear multiple regression analyses.

Results: FIM scores at Time 2 correlated with scores of a visual search test (Balloons Test B) at Time 1, namely completion time (r= –0.42, P=0.049), and targets identified at the left side of the page (r= –0.44, P=0.037) and that relative to the total targets identified on the whole page (r= –0.42, P=0.047). Stepwise regression analysis revealed that the number of targets identified at the left side of the page predicted positively the FIM at Time 2, R^2=0.191, F(1, 19)=4.496, P=0.047. GDS scores at Time 2 correlated with score on a verbal fluency test (r= –0.46, P=0.018), time to name colour patches as part of a Stroop test (r= 0.82, P=0.026), time to copy numbers according to symbols on the Symbol-Digit Modalities Test (r= –0.49, P=0.034), and immediate recall (r= –0.42, P=0.049) and delayed recall (r= –0.47, P=0.024) of Chinese Auditory Learning Test at Time 1. Stepwise linear multiple regression analysis revealed that time to name the colour patches at Time 2 predicted GDS at Time 2 even when GDS at Time 1 was controlled for, R^2=0.881, F(2, 4)=14.816, P=0.014.

Conclusion: Left visual selective attention and time to name colour patches at discharge from in-patient rehabilitation hospital predicted the stroke rehabilitation outcomes 3 months later.

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Multi-parameter isolation of primary lung cancer tumour stem cells (TSC); combining murine and human surface phenotyping in parallel with in-vivo tumour engraftment reveals multiple TSC phenotypes

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Introduction: There is emerging evidence that tumour comprises a hierarchy of biologically distinct cells. Tumour cells possessing the ability to regenerate growth have been termed tumour stem cells (TSC). TSC can be distinguished from non-tumourigenic population using surface phenotyping. The murine bronchoalveolar cancer stem cell has been defined as CD34+, 45-ve, 31-ve. CD133 had been reported as a TSC marker in human lung cancer. We postulate that there may be multiple cell surface markers defining TSC in lung cancer.

Methods: We prospectively collected surgical specimens from March 2007 to October 2008. Tumours are digested to single cells and then subjected to multi-parameter FACS phenotyping and/or xenograft formation assay in NOD-SCID or NOD-SCD IL2 gamma KO (NOG) mice. Xenograft formation indicating tumour-initiating cell activity is used as a surrogate for TSCs. Xenografts are serially passaged and subjected to further FACS assisted sorting to determine the surface phenotype of TSCs.

Results: Seven primary lung cancer specimens were collected; primary lung=61 (87%), LN=6 (9%), effusions=4 (6%), bone met=1 (1%). CD45+ haematopoietic cells comprises 55%±21 of all viable cells and CD45-ve cells comprises of 17%±11. After haematopoietic cells were excluded in phenotype analysis, the tumours cells can be fractioned to CD34+ 9.1%±12, CD133+ 11%±24, CD326+ 32%±27. Serially passaged tumours were re-digested and TSC activity determined by multi-parameter FACS sorting using CD133, CD34 and CD326 after exclusion of non-viable and murine cells. Of the xenografts produced (n=26), TSC activity does not rest exclusively in CD133+ fraction. Tumours can also arise from CD326+ and CD34+ fraction after concurrent negative gating for CD133.

Conclusion: We demonstrated the feasibility of high-resolution multi-parameter FACS analysis and sorting of primary lung cancer. Our data suggest the proportion of tumour cells within digests of primary lung cancers is no more than 20% of all viable cells. Our xenograft engraftment data suggest that human cells contribute to no more than 20% of all live cells obtained from xenograft experiments and CD133+ fraction is not the only one containing TSCs as previously reported in the literature.

Acknowledgement: This work was made possible by the STARR foundation US. I would also like to thank Drs M Moore and R Downey for their research support.

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Inactivation of Toll-like receptor 4 improves reendothelialisation in ApoE-deficient mice—impact of oxidative stress on endothelial progenitor cells

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Introduction: Atherosclerosis is an inflammatory disease which is in part mediated by Toll-like receptor 4 (TLR4). Endothelial injury, an initiating step in atherosclerosis, can be repaired through endothelial progenitor cell (EPC) activation, which has been shown to be diminished in patients with diabetes and/or vascular diseases. Vascular inflammation appears to impair the capacity of EPC in mediating the reendothelialisation process. It remains to be determined whether TLR4 is involved in this impairment.

Methods: ApoE-deficient (ApoEKO/TLR4WT) mice and ApoEKO mice lacking functional TLR4 (ApoEKO/TLR4KO) were used in this study. Wire injury was introduced to the right common carotid artery of the mice which were allowed to recover. Vascular repair was assessed by Evans blue staining of the injured arteries after 3 days. Circulating EPCs were quantified by flow cytometry analyses. Bone marrow–derived EPCs (BM-EPCs) were (1) assessed for reendothelialisation capacity after transplantation in vivo; (2) adhesion function in vitro; and (iii) reactive oxygen species production.

Results: (1) Reendothelialisation after wire injury was impaired in ApoEKO/TLR4WT mice and was associated with an increased number of circulating EPCs. Inactivation of TLR4 in ApoEKO/TLR4KO mice conferred an improved reendothelialisation, together with a paradoxical decrease in EPC number. Further findings suggested that the repair and adhesion capacity of EPCs from ApoEKO/TLR4WT mice was down-regulated. (2) Transplantation of BM-EPC isolated from ApoEKO/TLR4KO mice improved vascular repair, compared to cells from ApoEKO/TLR4WT mice. (3) Adhesion was diminished in BM-EPCs isolated from ApoEKO/TLR4WT mice and was normalised in BM-EPCs from ApoEKO/TLR4KO mice. (4) Oxidative stress was increased in BM-EPCs isolated from ApoEKO/TLR4WT mice, compared to those from ApoEKO/TLR4KO mice, suggesting a possible mechanism for EPC dysfunction.

Conclusion: Impaired reendothelialisation in atherosclerosis is attributable to a decline in EPC adhesion, which is reversible by TLR4 inactivation. Thus modulation of TLR4 activity may provide a mechanism to improve EPC function and hence protection against atherosclerosis.

Acknowledgement: This study was supported by a CRCG research grant, The University of Hong Kong, to FYL Li.

Non-genetic, mechanism-based facilitation of cardiomyogenesis and maturation from pluripotent human stem cells

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Recent studies have demonstrated that human embryonic stem cells can be efficiently and reproducibly directed into cardiomyocytes (CMs) using stage-specific induction protocols. Here we show that CMs derived from pluripotent human stem cells commonly displayed immature, pro-arrhythmic properties found in adult CMs from heart failure patients. By first identifying the absence of the inwardly rectifying K^+ current (I_{K1}), among the panoply of sarcolemmal ionic currents investigated ($I_{Na}^+/I_{CaL}^+/I_{Kr}^+/I_{NCX}^+/I_f^+/I_{to}^+/I_{K1}^-/I_{Ks}^-$), as the single mechanistic contributor to the failing-like properties, we rendered immature derived CMs adult-like by ablating such undesirable traits via somatic gene transfer of the missing ionic component. These results provided the first link of a complex developmentally arrested phenotype to a single effector gene, and importantly, further led us to develop a mechanism-based culturing strategy for enhancing cardiomyogenesis and driving global maturation. By providing the proper environmental cues, this approach did not require any genetic or pharmacological interventions. Our findings can facilitate clinical applications, drug discovery and cardiotoxicity screening by improving the yield, safety and efficacy of derived CMs.

Absence of transverse tubules contributes to non-uniform Ca^{2+} wavefronts in mouse and human embryonic stem cell–derived cardiomyocytes

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Mouse (m) and human embryonic stem cell–derived cardiomyocytes (hESC-CMs) are known to exhibit immature Ca^{2+} dynamics such as small whole-cell peak amplitude and slower kinetics relative to those of adult. In this study, we examined the maturity and efficiency of Ca^{2+}-induced Ca^{2+}-release in m and hESC-CMs, the presence of transverse (t)-tubules and its effects on the regional Ca^{2+} dynamics. In m and hESC-CMs, fluorescent staining and atomic force microscopy (AFM) were used to detect the presence of t-tubules, caveolin-3, amphiphysin-2 and colocalisation of dihydropyridine receptors (DHPRs) and ryanodine receptors (RyRs). To avoid ambiguities, regional electrically stimulated Ca^{2+} dynamics of single ESC-CMs, rather than spontaneously beating clusters, were measured using confocal microscopy. Mouse and hESC-CMs showed absence of dyads, with neither t-tubules nor colocalisation of DHPRs and RyRs. Caveolin-3 and amphiphysin-2, crucial for the biogenesis of t-tubules with robust expression in adult CMs, were also absent. Single m and hESC-CMs displayed non-uniform Ca^{2+} dynamics across the cell that is typical of CMs deficient of t-tubules. Local Ca^{2+} transients exhibited greater peak amplitude at the peripheral than the central region for m (3.50±0.42 vs 3.05±0.38) and hESC-CMs (2.96±0.25 vs 2.72±0.25). Kinetically, both the rates of rise to peak amplitude and transient decay were faster for the peripheral relative to the central region. Immature m and hESC-CMs display unsynchronised Ca^{2+} transients due to the absence of t-tubules and gene products crucial for their biogenesis. Our results provide insights for driving the maturation of ESC-CMs.

Overexpression of HCN-encoded pacemaker current silences bioartificial pacemakers

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Introduction: Current strategies of engineering bioartificial pacemakers from otherwise silent yet excitable adult atrial and ventricular cardiomyocytes primarily rely on either maximising the hyperpolarisation-activated I_f or on minimising its presumptive opponent, the inwardly rectifying potassium current I_{K1}. The purpose of this study was to determine quantitatively the relative current densities of I_f and I_{K1} necessary to induce automaticity in adult atrial cardiomyocytes.

Methods: Automaticity of adult guinea pig atrial cardiomyocytes was induced by adenovirus (Ad)-mediated overexpression of the gating-engineered HCN1 construct HCN1-DeltaDeltaDelta with the S3-S4 linker residues EVY235-7 deleted to favour channel opening.

Results: Whereas control atrial cardiomyocytes remained electrically quiescent and had no I_f, 18% of Ad-CMV-GFP-IRES-HCN1-DeltaDeltaDelta (Ad-CGI-HCN1-DeltaDeltaDelta)–transduced cells demonstrated automaticity (240±14 bpm) with gradual phase-4 depolarisation (143±28 mV/s), a depolarised maximal diastolic potential (-45.3 ±2.2 mV), and substantial I(f) at -140 mV ($I_{f,-140\ mV}$= –9.32±1.84 pA/pF). In the remaining quiescent Ad-CGI-HCN1-DeltaDeltaDelta–transduced atrial cardiomyocytes, two distinct immediate phenotypes were observed: (1) 13% had a hyperpolarised resting membrane potential (-56.7±1.3 mV) with $I_{f,-140\ mV}$ of -4.85±0.97 pA/pF; and (2) the remaining 69% displayed a depolarised resting membrane potential (-27.6±1.3 mV) with I(f,-140 mV) of -23.0± 3.71 pA/pF. Upon electrical stimulation, both quiescent groups elicited a single action potential with incomplete phase-4 depolarisation that was never seen in controls. Further electrophysiologic analysis indicates that an intricate balance of I_{K1} and I_f is necessary for induction of atrial automaticity.

Conclusion: Optimised pacing induction and modulation can be better achieved by engineering the I_f/I_{K1} ratio rather than the individual currents.

Toll-like receptor 4 mediates tubular inflammation in diabetic nephropathy

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Introduction: Toll-like receptor 4 (TLR4) has been implicated in the regulation of immune responses and inflammatory disease. Diabetic nephropathy is being increasingly recognised to comprise a heavy inflammatory element that results in tubulointerstitial lesions, which govern overall renal prognosis. However, the role of TLR4 in tubulointerstitial injury during diabetic nephropathy is still unknown.

Methods: Human proximal tubular epithelial cells (PTEC) were employed to examine the effect of high glucose (HG) on TLR4 expression. The Ikappa B/NF-kappa B activation was examined by western blot and ELISA. TLR4 content was detected by immunohistochemistry in nine human renal biopsies with histologically proven diabetic nephropathy.

Results: HG induced TLR4 overexpression in PTEC in a time- and dose-dependent manner, resulting in upregulation of IL-6 mRNA via Ikappa B/NF-kappa B activation. Blockade of TLR4 in PTEC by pre-incubation with a neutralising antibody resulted in a significant decrease in HG-induced Ikappa B/NF-kappa B activation, and the associated downstream IL-6 synthesis. Immunohistochemical analyses of human renal biopsies revealed that TLR4 were expressed in proximal and distal tubules, with more intense staining in kidneys with histologically proven diabetic nephropathy compared with normal controls.

Conclusion: Our findings suggest a novel TLR4-mediated pathway through which hyperglycaemia may contribute to tubular inflammation in the diabetic kidney.

Thymomatous myasthenia gravis

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Background: Myasthenia gravis (MG) is an autoimmune disorder targeting skeletal muscle acetylcholine receptor. Thymoma is associated with MG in some patients, the majority of whom present with symptoms of MG before detection of underlying thymoma. This study aimed to study clinical and serological characteristics of Chinese thymomatous MG patients.

Methods: Autoimmune MG patients with thymomectomy and histologically confirmed thymoma followed up in our hospital for at least 12 months were studied.

Results: A total of 37 Chinese MG patients with histology-proven thymoma were retrospectively studied. The mean MG symptom onset age was 48.5 (range, 25-81) years; 25 (68%) were female. The mean follow-up duration was 4.9 (range, 1-15) years. Symptoms of MG preceded detection of thymoma in the majority (31 patients, 84%), in six patients thymoma detection preceded MG symptoms onset by 1 to 8 years. Nineteen (51%) patients had early-onset MG (before 50 years of age). All patients were seropositive for acetylcholine receptor antibodies and 30 (81%) patients seropositive for striated muscle antibodies. Eleven (30%) patients had experienced myasthenic crisis and the worst MGFA clinical severity grade were class I (6 patients), class II (3), class III (8), class IV (9), and class V (11); hence 31 (84%) had generalised MG and six (16%) had ocular MG. Twenty-seven (73%) patients had a history of corticosteroid therapy, 22 (60%) required azathioprine, two required other immunosuppressant (1 mycophenolate mofetil, 1 cyclosporin A). All 37 patients had good or satisfactory MG clinical outcome measured by MGFA post-intervention status (2 pharmacological remission, 23 minimal manifestation, 12 improved) though one patient died from metastatic thymoma.

Conclusion: Thymoma MG was clinically severe with frequent myasthenic crises, but responses to conventional immunosuppressive therapies are satisfactory.

Understanding molecular mechanisms underlying Lrrk2-related Parkinson's disease in mouse

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Parkinson's disease (PD) is the second most common neurodegenerative movement disorder, characterised by slow movement, resting tremor, and postural instability. *Lrrk2* has been added to the list of genes that are implicated in PD. LRRK2 is a very large gene, which has over 2527 amino acid and 51 exons. LRRK2 is a complex protein consisting of five domains, which is expressed throughout the brain and the whole body. More than 20 mutations have been reported in LRRK2 in PD patients in different races. More importantly, 5 to 6% of familial PD patients have these mutations. Some in these mutations, R1441C, R1441G, Y1699C and G2019S, are amino acids conserved across vertebrates. Some patients with LRRK2 mutations have loss dopamine neuron in substantia nigra and Lewy body, which are typical features of PD. Initial studies proved these mutations increased LRRK2 kinase activity with autophosphorylation. Tauopathy and hyperphosphorylated tau are also found in Lrrk2 in vitro models. All these indicated its important role in PD pathogenesis. The importance of the R1441 residue in the pathogenesis is highlighted by the identification of three distinct missense mutations. We generated R1441G KI mice, did molecular characterisation of it, and analysed normal DA neurons change in R1441G KI mice after MPTP injection. Furthermore, we compared the mitochondrial parameters difference between wild type and single mutation proteins (R1441G R1441C and G2019S) in HEK293 and Sh-Sy5y cell lines.

Acknowledgements: I am grateful to my Supervisor Prof Ho and Co-Supervisor Dr Zhou. I thank all my Labmates for their many valuable suggestions.

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Functional sarcoplasmic reticulum for calcium handling of human embryonic stem cell–derived cardiomyocytes: insights for driven maturation

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Cardiomyocytes (CMs) are non-regenerative. Self-renewable pluripotent human embryonic stem cells (hESCs) can differentiate into CMs for cell-based therapies. In adult CMs, Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR) via the ryanodine receptor (RyR) is key in excitation-contraction coupling. Therefore, proper Ca^{2+} handling properties of hESC-derived CMs are required for their successful functional integration with the recipient heart. Here, we performed a comprehensive analysis of CMs differentiated from the H1 (H1-CMs) and HES2 (HES2-CMs) hESC lines and human foetal (F) and adult (A) left ventricular (LV) CMs. Upon electrical stimulation, all of H1-, HES2-, and FLV-CMs generated similar Ca^{2+} transients. Caffeine induced Ca^{2+} release in 65% of FLV-CMs and approximately 38% of H1- and HES2-CMs. Ryanodine significantly reduced the electrically evoked Ca^{2+} transient amplitudes of caffeine-responsive but not -insensitive HES2- and H1-CMs and slowed their upstroke; thapsigargin, which inhibits the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pump, reduced the amplitude of only caffeine-responsive HES2- and H1-CMs and slowed the decay. SERCA2a expression was highest in ALV-CMs but comparable among H1-, HES2-, and FLV-CMs. The Na^{+}-Ca^{2+} exchanger was substantially expressed in both HES2- and H1-CMs relative to FLV- and ALV-CMs. RyR was expressed in HES2-, H1-, and FLV-CMs, but the organised pattern for ALV-CMs was not observed. The regulatory proteins junctin, triadin, and calsequestrin were expressed in ALV-CMs but not HES2- and H1-CMs. We conclude that functional SRs are indeed expressed in hESC-CMs, albeit immaturely. Our results may lead to driven maturation of Ca^{2+} handling properties of hESC-CMs for enhanced contractile functions.

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The protein kinase Akt regulates the intracellular localisation of LKB1 by phosphorylation-dependent interaction with 14-3-3

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Introduction: LKB1, a tumour suppressor protein kinase causally linked to the Peutz-Jeghers syndrome, is a key player in both energy metabolism and cell proliferation. Several previous studies demonstrated that the intracellular localisation of LKB1 is critical for its functions. However, the precise mechanism that controls the subcellular distribution remains elusive. Our previous mass spectrometry analysis showed that serine^{334} on LKB1 is phosphorylated. Scansite prediction showed that this site is located in both the recognition motif of 14-3-3 and the phosphorylation site of Akt. The objectives of this study were to investigate whether LKB1 is a downstream target of Akt and a novel binding partner of 14-3-3, and to elucidate how the intracellular localisation and functions of LKB1 can be regulated by Akt and 14-3-3.

Methods: Seven GST-tagged 14-3-3 isoforms purified from *E coli* were used for pull-down experiment to check whether 14-3-3s interact with LKB1. Akt inhibitors, Akt constitutively active (CA) and dominant negative (DN) adenovirus were used to evaluate whether the interaction between LKB1 and 14-3-3 can be regulated by LKB1 phosphorylation. In-vitro phosphorylation assay, mass spectrometry (MS) analysis and site-directed mutagenesis were employed to determine whether serine^{334} is both the phosphorylation site of Akt and 14-3-3 binding site.

Results: Western blot analysis showed that five of the seven 14-3-3 isoforms interacted with LKB1. Pharmacological or molecular inhibition of the Akt activity resulted in both the decreased interaction between 14-3-3 zeta and LKB1 as well as the increased cytosolic/nuclear ratio of LKB1 which ultimately can lead to the decreased LKB1 protein stability due to the cytosolic proteasome–mediated degradation. In-vitro phosphorylation assay result confirmed that LKB1 is a direct target of Akt and the subsequent MS analysis demonstrated that the precise phosphorylation site is on serine^{334}. Site-directed mutagenesis revealed that mutating serine^{334} of LKB1 to alanine led to both the decreased interaction between LKB1 and 14-3-3zeta and increased cytosolic localisation.

Conclusions: LKB1 is a downstream target of the protein kinase Akt and a novel interaction partner of 14-3-3. Akt induces the phosphorylation of LKB1 at serine^{334}, and enhances the interaction of LKB1 with 14-3-3. Inhibition of Akt activity leads to the translocation of LKB1 from nuclei to cytosol for proteosome-mediated degradation. These findings identified a new pathway underlying the cross-talk between Akt and LKB1, the two important kinases involved in both metabolism and cell growth.

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

Does vocational training in family medicine have an impact on general practice patterns?

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Introduction: The focus of health care system around the world is shifting towards primary care and better primary care systems have better health outcomes. Vocational training (VT) in family medicine has advanced over the last decade, but little is known about the practice patterns of such vocationally trained doctors. This study was to determine whether VT of family physicians (FPs) has any effect on their practice patterns.

Methods: Members of the Hong Kong College of Family Physicians were invited to take part in a year-long territory-wide primary care morbidity and management survey. They used standardised data collection forms and prospectively recorded all health problems and management activities during selected weeks of data collection. Characteristics of VT and non-VT FPs, morbidity pattern of their patients, and their management activities were compared using regression analyses.

Results: A total of 109 FPs took part in the survey; 67 had VT and contributed to 55.3% of all patient encounters (n=52 337). Compared with non-VT FPs, VT FPs looked after more patients with chronic diseases. They prescribed fewer drugs per encounter (odds ratio= –0.23, P<0.05) and were less likely to prescribe antibiotics (odds ratio=0.68, P<0.05), non-benzodiazepine hypnotics (odds ratio=0.46, P<0.05) and anti- depressants (odds ratio=0.622, P<0.05). However they ordered more investigation (odds ratio=2.01, P<0.05) and made more referrals (odds ratio=1.79, P<0.05).

Conclusion: VT in family medicine has significant association with patient management. Our study shows that FPs with VT took care of more patients with chronic diseases and were more vigilant in prescribing. VT in family medicine should be supported and the family doctor concept promoted to help strengthen the primary care system.

Patients with and without family doctors—what differences were there in health care?

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Introduction: Primary care systems in many Asian populations like Hong Kong do not commonly share the family doctor concept. People are free to choose their own primary care doctors. However, the quality and effectiveness of such consultations are unknown. This study evaluated the difference in the quality of primary care consultations between people with and without a family doctor.

Methods: Cross-sectional telephone survey on the general population of Hong Kong using structured questionnaire to collect information on patient reported outcomes (global rating of change in health, enablement to cope with illness using Patient Enablement Instrument [PEI]) and various outcome indicators of quality of consultations.

Results: Compared with participants having no regular doctors (NRD, n=619) and those having regular non-Family Medicine doctors (RnFD, n=418), participants with regular family doctors (RFD, n=538) had global improvement in health after consultation (mean, 0.98 vs 0.76, 0.76; P<0.05), higher PEI scores (mean, 3.0 vs 2.2, 2.3; P<0.05) and were more likely to consult for preventive care (odds ratio=1.87, 1.37, P<0.05). RFD and RnFD were more likely than NRD to have blood pressure measured within a year (odds ratio=1.81, 1.78; P<0.05). RFD were more likely than NRD to have had cervical cancer screening (odds ratio=1.73, P<0.05) and to consult for chronic diseases (odds ratio=1.50, P<0.05). NRD and RnFD had more illness episodes than RFD (coefficient=0.31, 0.54, P<0.05) and were more likely to attend emergency department during the last illness episode (odds ratio=2.31, 2.01; P<0.05).

Conclusion: Participants with RFD reported better outcomes and received more comprehensive care than those without. This provides evidence for promoting a family doctor–led model primary care system in Asia.

Effect of infliximab on cardiovascular risk profile in patients with psoriasis: a pilot study

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Introduction: Epidemiological evidence suggested that patients with psoriasis have an increased risk of cardiovascular diseases. Anti-TNFα agents had been used with good efficacy in disease control in psoriasis. The aim of this study was to assess whether infliximab modified the cardiovascular risk profile in psoriasis.

Methods: Cardiovascular risk profile was measured before, during, and after treatment in 10 patients with psoriasis receiving a 24 weeks' course of infliximab. Assessment included: (1) panel of inflammatory markers and cytokines (lipid and apolipoprotein profile, adiponectin, leptin, IFNγ, TNFα, IL-1b, IL2, IL4, IL5, IL6, IL8 and IL10); (2) intimal medial thickness; (3) reactive hyperaemic index for endothelial function; (4) pulse wave velocity for arterial stiffness; and (5) ankle brachial index. Severity of psoriasis was measured by Psoriasis Area and Severity Index (PASI) and Nail Psoriasis Severity Index (NAPSI).

Results: There was significant improvement in disease control (PASI=75 in 67%, mean NAPSI reduction of 58%) at week 24. Treatment was complicated by pulmonary tuberculosis in one patient. There was significant reduction of IFNγ from $0.6x10^{-3}$ to $0.2x10^{-3}$ ($P<0.05$), IL17 from 46 pmol/mL to 44 pmol/mL ($P<0.05$), TNFα from $14x10^{-3}$ to $7x10^{-3}$ ($P<0.05$) and leptin of 0.87 ng/mL (0.03-1.72 ng/mL) at week 24. However, no significant differences were noted in intimal medial thickness, reactive hyperaemic index, pulse wave velocity, and ankle brachial index.

Conclusion: Infliximab was an effective treatment for patients with psoriasis. Apart from the reduction of TNFα, its effect may mediate through the reduction of signature cytokines of T helper 1 (IFNγ) and T helper 17 (IL17) cells. The preliminary result did not show significant improvement in cardiovascular risk profile after 6 months of infliximab therapy in psoriasis.

Rehabilitation outcomes 6 months after discharge from the Geriatric Day Hospital in older Chinese patients

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Introduction: Whether rehabilitation outcome can be maintained after discharged from Geriatric Day Hospital (GDH) has not been thoroughly investigated. This study was conducted to examine the rehabilitation outcome and its predictors 6 months after discharged from GDH.

Methods: It was a retrospective study performed in the GDH of Fung Yiu King Hospital. Cognitive status was assessed with Cantonese version of Mini-Mental State Examination (C-MMSE). Functional Independence Measure (FIM) upon GDH admission (FIM admission), discharge (FIM discharge), and 6 months after discharge (FIM post 6 months) were measured. FIM efficacy was FIM discharge – FIM admission, while FIM efficiency was FIM efficacy divided by number of GDH visits.

Results: A total of 418 patients attended post 6 months GDH assessment between January 2005 and December 2007 were studied. In all, 164 (39.2%) showed a drop of FIM after 6 months. There was a significant drop of FIM post 6 months as compared with FIM discharge (91.5±17.1 vs 93±20.5, $P<0.001$). However, the FIM post 6 months remained significantly higher than FIM admission (91.5±17.1 vs 86.8±17.4, $P<0.001$). Univariate analysis showed that living in old-age home, incontinence, having musculoskeletal problems or Parkinsonism as the main complaint, C-MMSE score, FIM admission, FIM discharge and FIM efficiency were significant factors related to FIM drop post 6 months. Multivariate analysis revealed that FIM discharge was a negative predictor (odds ratio=0.96; 95% CI, 0.95-0.98; $P<0.001$) while Parkinsonism was a positive independent predictor for FIM drop post 6 months (odds ratio=3.2; 95% CI, 1.35-7.5; $P=0.008$).

Conclusion: A proportion of functional gain can still be maintained 6 months after discharged from GDH. More studies are needed to look for strategies in maintaining functional gain in GDH discharged patients, especially those with Parkinsonism.

Gender differences in rehabilitation outcomes among older Chinese patients

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Introduction: At present, male and female patients undergo the same programme in geriatric rehabilitation. During literature review, we cannot find any study concerning whether there are gender differences in geriatric rehabilitation. This study investigated the relationship between gender and rehabilitation outcomes of older Chinese patients.

Methods: It was a retrospective study carried out in two geriatric rehabilitation hospitals in Hong Kong. Absolute functional and motor gains were expressed as Barthel Index (BI) efficacy and Elderly Mobility Scale (EMS) efficacy. BI and EMS efficiency were efficacy divided by the length of stay. Satisfactory motor and functional outcomes were defined as discharge EMS ≥15 and BI ≥75.

Results: A total of 1795 patients were studied. Compared with men, women had higher BI but lower EMS on admission and discharge. EMS and BI efficacy and efficiency were similar in both sexes. Female gender was a significant independent negative predictor for satisfactory motor outcome (P=0.0002) but a positive predictor for functional outcome (P=0.0007). Other independent predictors for satisfactory motor outcome were: age (P<0.001); urinary incontinence (P=0.0049); living at home (P=0.0056); admission EMS (P<0.001); admission BI (P=0.044). Other predictors for satisfactory functional outcome were: age (P=0.009); infection other than chest (P=0.047); urinary incontinence (P<0.001); Mini-Mental State Examination (P=0.0004); admission EMS (P=0.005); BI (P<0.001).

Conclusion: Women achieved a better functional outcome but a poorer motor outcome on discharge. Female gender was a positive predictor for functional outcome but a negative factor for motor outcome. Our results seem to suggest that a gender-tailored rehabilitation programme is needed to foster the motor and functional independence of older men and women.

Distinctive functions of methionine aminopeptidase II in embryonic haematopoiesis in zebrafish embryos

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Introduction: Methionine aminopeptidase 2 (MetAP-2) is known to be the target of an anti-angiogenesis compound fumagillin and has been investigated for its robust expression in human cancers and its anti-proliferative effects on endothelial cells. Together with a related member MetAP-1, they are proteases which remove the initiator NH2-terminal methionine from the nascent peptides during protein translation. Despite its pathogenetic significance in cancers, the physiological role of MetAP-2, particularly during embryonic development, has remained unclear. In this study, we made use of the zebrafish model and investigated the expression and functions of MetAP-2 during embryonic development, with particular reference to haematopoiesis.

Methods and Results: We injected a morpholino (4.5 ng) targeting at the splice-site junction of MetAP-2 gene into zebrafish embryos at 1-4 cell stage (referred as MetAP-2^{MO} embryos). Molecular targeting was confirmed by real-time polymerase chain reaction (RT-PCR). Modulation of MetAP-2 activity was shown by a shift of isoelectric point of GAPDH in 2-dimensional electrophoresis. The MetAP-2^{MO} embryos exhibited a kinked tail with altered somitic patterning and specific changes in haematopoietic gene expression as shown by both whole-mount in-situ hybridisation and RT-PCR. The latter included down-regulation of *scl* and *lmo2* (primitive HSC) and up-regulation of *mpo* (primitive myeloid cells) at 18 hpf, as well as up-regulation of *c-myb* and *runx1* (definitive HSC) at 36 hpf. Importantly, the haematopoietic phenotype could be rescued by co-injecting the embryos with MetAP-2 mRNA and re-capitulated by treating the embryos with fumagillin and its analogues. Mechanistically, the MetAP-2^{MO} embryos exhibited reduced camodulin kinase II (CaMKII) activity and the haematopoietic phenotypes could be rescued by CaMKII mRNA and recapitulated by a CaMKII inhibitor. Treating the embryos with inhibitors of RhoA and JNK also recapitulated some of the haematopoietic phenotypes. The canonical Wnt pathway, characterised by β-catenin signalling, was not affected.

Conclusion: We demonstrated that MetAP-2 knock-down resulted in down-regulation of *scl* and *lmo2* and up-regulation of *mpo*, *c-myb* and *runx1*. Treating the embryos with a specific MetAP-2 inhibitor fumagillin and its structural analogues recapitulated these changes in haematopoietic gene expression, supporting the proposition that MetAP-2 is involved in the regulation of embryonic haematopoiesis. Mechanistically, the haematopoietic phenotypes were linked to the modulation of non-canonical but not the canonical Wnt pathways. These observations have provided us with important insights to the regulation of embryonic haematopoiesis.

The effect of diethylaminobenzaldehyde, an inhibitor of aldehyde dehydrogenase, on primitive haematopoiesis during zebrafish embryonic development

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Introduction: Aldehyde dehydrogenase (Aldh) are a group of enzymes involved in the biosynthesis of retinoic acid as well as the metabolism of amino acid, fatty aldehydes, ethanol and cyclophosphamide. Despite the widely reported expression in primitive haematopoietic stem and progenitor cells (HSPC), its function during haematopoiesis was unclear. Inhibition of Aldh with diethylaminobenzaldehyde (DEAB) in vitro delays differentiation and expand human HSPC due to inhibition of retinoic acid biosynthesis. In this study, we examine the effect of DEAB in vivo on zebrafish embryos with particular reference to its effect in primitive haematopoiesis.

Methods: Wild-type and transgenic [Tg(gata1:gfp), Tg(fli1:gfp)] embryos were treated with DEAB (1 μmol/L) between 1-cell to long-pec stage. Treated embryos were evaluated in terms of morphology, flow cytometry, in-situ hybridisation (ISH) and Q-RT-PCR.

Results: At 36 hpf, the intermediate cell mass where primitive haematopoiesis happens was significantly expanded without detectable vascular abnormality. Genes associated with HSC (*scl*, *lmo2*), erythropoiesis (*gata1*, and *embryonic hemoglobins*) and myelopoiesis (*spi1*) were also significantly up-regulated as shown by ISH and Q-RT-PCR. Upon DEAB treatment, there was a significant increase in GFP^+ cells (representing erythroid cells) in Tg(gata1:gfp) embryos, which could be ameliorated by concomitant treatment of all-trans retinoic acid (ATRA) [control: 4.39±0.11%; DEAB: 6.15±0.29%; DEAB+ATRA: 4.46±0.12%; P=0.012].

Conclusion: DEAB treatment induces expansion in primitive haematopoiesis in zebrafish embryos probably through the inhibition of Aldh and retinoic acid synthesis.

Intracerebral haemorrhage complicating anticoagulant therapy among Hong Kong Chinese

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Background: Anticoagulation is effective to prevent cardioembolism in patients with atrial fibrillation (AF) and prosthetic heart valves, but carries risk of potentially life-threatening intracerebral haemorrhage (ICH). The ideal international normalised ratio (INR) for Chinese patients on warfarin treatment is uncertain. We aimed to study the clinical and radiological characteristics of Chinese patients who developed acute ICH while on warfarin.

Methods: Patients with diagnostic code of ICH from January 2000 to December 2008 were reviewed, those who had ICH while taking warfarin were studied.

Results: Among 1114 patients with ICH, 54 patients had 58 episodes of ICH while taking warfarin. Four patients were excluded due to inadequate data, 50 patients (31 for AF, 14 for prosthetic heart valves, 5 for DVT) with 54 ICH episodes were studied. Their mean age was 72.2 (range, 34-94) years, 25 (50%) were male. Their mean INR on presentation with ICH was 2.6 (range, 1.2-6.8). Sites of ICH revealed on CT scan were supratentorial (32), infratentorial (17), multifocal (2), intraventricular haemorrhage (2), and unknown (1). The mean Glasgow Coma Scale score (GCS) at presentation was 12.26 (range, 3-15). Importantly, 27 (50%) of the 54 ICH episodes resulted in mortality; among the 27 ICH episodes that did not result in mortality, 23 (42.6%) episodes had good neurological recovery and 4 (7.4%) episodes had poor recovery. *t* Test revealed that patients with poor outcome (defined as mortality, or neurological disability leading to ADL dependency/ADL requiring moderate assistance) had shorter duration of warfarin therapy (P=0.014), lower GCS at presentation (P=0.000), greater pulse pressure at presentation (P=0.022), higher pulse rate at presentation (P=0.028), higher mean systolic BP on day 1 (P=0.018) but lower mean systolic BP (P=0.001), mean diastolic BP (P=0.018) and mean arterial pressure (P=0.001) on day 2, greater BP fluctuations on day 1 (P=0.001), and higher white blood count at presentation (P=0.015) than patients with good outcome (ADL independent/requiring mild assistance). Logistic regression analysis revealed that presence of intraventricular extension (P=0.000, OR=28.8), GCS <12 on presentation (P=0.005, OR=20.6) and smoking (P=0.047, OR=5.3) predicted poor clinical outcome.

Conclusion: Warfarin-related ICH accounted for approximately 5% of ICH in this hospital-based study and occurred with a mean INR of 2.6. It is a serious condition with mortality rate of 50%. INR on presentation did not affect clinical outcome.

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The relation of cytokines of IL-17/IL-23 axis to Th1/Th2 cytokines and disease activity in systemic lupus erythematosus

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Introduction: Interleukin (IL)-17 is recently linked to the pathogenesis of systemic lupus erythematosus (SLE) but its relation to disease activity has not been well characterised. The objectives of this study were to examine the relation of serum cytokine levels from the IL-17/IL-23 axis (IL-17, IL-23) to Th1 (IL-12, IFN-γ), Th2 (IL-10, IL-6, IL-4) cytokines and disease activity in SLE patients.

Methods: Serum cytokines were measured by enzyme-linked immunosorbent assays. Disease activity was determined by SLE disease activity index (SLEDAI), anti-dsDNA antibody, C3 and C4 levels.

Results: Serum levels of IL-17, IL-10 and IFN-γ were higher in SLE patients (n=70) compared to age- and sex-matched controls (n=14) [P<0.001]. Higher serum IL-23 level was found in active lupus patients who had cutaneous manifestation (P=0.003) and serositis (P=0.03) compared to those who had not. Serum IL-17 was not different between patients who had active lupus nephritis (n=23), non-renal active lupus (n=13) and inactive disease (n=34) [P=0.23]. However, an inverse correlation between serum IL-17 with proteinuria was found among all SLE patients (r= –0.27, P=0.03). Serum IL-17 level was, otherwise, not related to SLEDAI, glomerular filtration rate, activity or chronicity score and ISN/RPS class among patients with active lupus nephritis and was not found to correlate with serum IFN-γ or IL-10.

Conclusions: Elevated serum IL-23 was found in patients with inflammatory manifestations including cutaneous involvement and serositis. Serum IL-17 level was not shown to correlate with disease activity but demonstrated an inverse correlation with proteinuria suggesting urinary loss of IL-17 and its involvement in lupus renal pathology.

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Serum levels of IL-33 and soluble ST2 and their association with disease activity in systemic lupus erythematosus

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Background: IL-33 has recently been found to be the specific ligand of ST2, an IL-1 receptor family member that is selectively expressed on Th2 cells and mediates Th2 response. This study aimed to measure serum levels of soluble form of ST2 (sST2) and IL-33 in patients with systemic lupus erythematosus (SLE) and to examine its association with disease activity.

Methods: Seventy SLE patients were evaluated for disease activity determined by SLE disease activity index (SLEDAI), serological features (anti-dsDNA antibody, C3 and C4) and 57 patients were evaluated longitudinally on a second occasion. IL-33 and sST2 were measured by sandwich ELISA in the 127 SLE serum samples and compared to 28 age- and sex-matched healthy controls.

Results: Serum sST2 level was significantly higher in SLE patients with active disease (0.51+0.18 ng/mL) compared to those with inactive disease (0.42+0.08 ng/mL) [P=0.006] and to normal controls (0.36+0.13 ng/mL) [P<0.001]. sST2 level correlated significantly and positively with SLEDAI, level of anti-dsDNA antibody and prednisolone dosage and negatively with C3 and remained significantly predictive of active disease after adjustment for prednisolone use in logistic regression analysis (odds ratio=4.6, P=0.01). sST2 level was sensitive to change in disease activity in longitudinal evaluation and not influenced by age, gender, and renal function. Elevated serum IL-33 was comparable in frequency (4.3% vs 7.1%, P=0.62) and levels (P=0.53) between SLE patients and controls.

Conclusion: Elevated serum sST2 level in SLE patients was found to correlate with disease activity and was sensitive to change, suggesting a potential role as surrogate marker of disease activity.

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Southern Chinese patients with systemic lupus erythematosus in Hong Kong have low vitamin D levels

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Background: Vitamin D insufficiency has been linked to pathogenesis of autoimmune diseases. This study aimed to measure serum 25(OH)D level in patients with systemic lupus erythematosus (SLE) in Hong Kong and to evaluate association between serum 25(OH)D level and disease activity.

Methods: Serum 25(OH)D level was measured by radioimmunoassay in SLE patients and healthy controls. Lupus disease activity was determined by SLE disease activity index (SLEDAI), serum anti-dsDNA antibody, C3 and C4 levels.

Results: Fifty-two SLE patients with mean ± standard deviation disease duration of 15.5±8.6 years were recruited. Five patients had active lupus disease. Five (9.6%) patients had serum 25(OH)D levels <30 nmol/L. Serum 25(OH)D level was significantly lower in SLE patients compared to age- and sex-matched controls (n=52) [45.5±12.3 vs 51.1±12.6 nmol/L, P=0.02]. Serum 25(OH)D levels were not found to be related to SLEDAI, elevated anti-dsDNA antibody, low C3 or C4 levels or medications. One vitamin D insufficient patient had low serum albumin-corrected calcium. Serum 25(OH)D levels were found to correlate negatively with estimated glomerular filtration rate (r= –0.30, P=0.03) but was not different between patients who had normal and impaired renal function (P=0.38).

Conclusion: SLE patients in Hong Kong were found to have low serum 25(OH)D level despite its subtropical location.

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Functional consequences of overexpressing the gap junction Cx43 in the cardiogenic potential of pluripotent human embryonic stem cells

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Gap junctions, encoded by the connexin (Cx) multi-gene family, couple adjacent cells and underlie cell-cell communications. Previous mouse studies suggest that Cxs play an important role in development but their role in human cardiogenesis is undefined. Human embryonic stem cells (hESC) provide a unique model for studying human differentiation. Lentivirus-mediated stable overexpression of Cx43 in hESC (Cx43-hESC) did not affect colony morphology, karyotype and expression of pluripotency genes such as Oct4 but completely suppressed the formation of spontaneously beating, cardiomyocyte-containing clusters in embryoid bodies (EBs). Unlike control hEBs, the transcripts of several mesodermal markers (kallikrein, delta-globin, and CMP), ventricular myosin light chain and cardiac troponin I were absent or delayed. Transcriptomic and pathway analyses showed that 194 genes crucial for movement, growth, differentiation and maintenance were differentially expressed in Cx43-hESC. We conclude that Cx43 mediates the expression of an array of genes involved in human cardiogenesis, in addition to intercellular communication.

Distinct cardiogenic preferences of two human embryonic stem cell lines are imprinted in their proteomes in the pluripotent state

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Although both the H1 and HES2 human embryonic stem cell (hESC) lines (NIH codes: WA01 and ES02, respectively) are capable of forming all three germ layers and their derivatives, various lines of evidence including the need to use different protocols to induce cardiac differentiation hint that they have distinct preferences to become chamber-specific heart cells. However, a direct systematic comparison has not been reported. Here we electrophysiologically demonstrated that the distributions of ventricular-, atrial- and pacemaker-like derivatives were indeed different (ratios=39:61:0 and 64:33:3 for H1 and HES2, respectively). Based on these results, we hypothesised the differences in their cardiogenic potentials are imprinted in the proteomes of undifferentiated H1 and HES2. Using multiplexing, high-resolution 2-D Differential In Gel Electrophoresis (DIGE) to minimise gel-to-gel variations that are common in conventional 2-D gels, a total of 2000 individual protein spots were separated. Of which, 55 were >2-fold differentially expressed in H1 and HES2 ($P<0.05$) and identified by mass spectrometry. Bioinformatic analysis of these protein differences further revealed candidate pathways that contribute to the H1 and HES2 phenotypes. We conclude that H1 and HES2 have predetermined preferences to become ventricular, atrial, and pacemaker cells due to discrete differences in their proteomes. These results improve our basic understanding of hESCs and may lead to mechanism-based methods for their directed cardiac differentiation into chamber-specific cardiomyocytes.

Activation of endothelial nitric oxide synthase is required for hypoxia inducible factor (HIF)–1α–mediated cardiac differentiation of embryonic stem cells

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Introduction: We have previously showed that the activation of hypoxia inducible factor (HIF)–1 pathway promotes cardiac differentiation of cultured embryonic stem cells (ESCs). This study investigated the role of endothelial nitric oxide synthase (eNOS) activation in the HIF-1α induced cardiac differentiation of ESCs.

Methods: Mouse ESCs were transduced with lentivirus containing HIF-1α cDNA and subjected to cardiac differentiation using the 'hanging drop method'. If necessary, 100 nM of diphenyleneiodonium chloride (DPI) was added to the culture medium to inhibit the eNOS system. The phosphorylation of eNOS was evaluated by detecting western blot analysis. The cardiac differentiation was evaluated by the yield of beating embryoid bodies (EB), flow cytometry analysis on the troponin-T positive cells and real-time quantitative PCR analysis of the expressions of cardiac markers.

Results: Overexpression of HIF-1α remarkably increased the serine 1171 phosphorylation of eNOS is the mouse ESCs. Accompanied with this, the yield of beating EB and troponin-T positive cells significantly increased by about 2 and 3 folds respectively in the HIF-1α transduced group. On the other hand, application of 100 nM DPI to the transduced cells completely abolished the HIF-1α induced increase in the yield of beating EB and troponin-T positive cells.

Conclusion: Taken collectively, our results demonstrated the importance of hypoxia in enhancing both cardiac differentiation and maturation of the cultured hESCs.

Acknowledgements: This work was supported by grants from RGC Grant (7769/08M to H.F.T.; C.W.S.; and R.A.L.), and the HKU Small Project Funding (200707176046 to K.M.N. , R.A.L., M.L.F. and H.F.T.)

Actopaxin: a novel regulator of cell migration and invasion in human hepatocellular carcinoma

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Introduction: The elementary steps leading to the development of metastasis is a complex process involving cell spreading, lamellipodia formation, and cell migration. Actopaxin, a focal adhesion and cytoskeleton-associated protein, is a member of a multi-gene family of which its phosphorylation at Ser/Thr-Pro motifs is required for such processes. The objective of this study was to examine the role of Actopaxin in hepatocellular carcinoma (HCC) cell migration, invasion and development of metastasis.

Methods: The expression of Actopaxin in primary and metastastic liver cancer cell lines was examined by western blot and RT-PCR. The functional effects of enforced expression or down-regulation of Actopaxin was investigated by cell migration, cell invasion, and wound healing assays. The expression of downstream signalling targets of Actpaxin and markers for epithelial mesenchymal transition was studied. Immunofluorescence staining was used to examine the effect of Actopaxin expression on cell shape, stress fibre organisation, and focal adhesion.

Results: The expression of Actopaxin was found to be highly expressed in the metastatic HCC cell lines H2M, MHCC-97L and MHCC-97H as compared with other primary HCC cell lines. Expression of a shorter form of Actopaxin (SF-Actopaxin) was also detected by western blot in some of these cell lines, suggesting the presence of more than one form of Actopaxin in human cells. The SF-Actopaxin lacks a fragment in the C-terminal, resulting in an incomplete second CH domain which consists of binding sites for its downstream activation targets. Enforced expression of long-form Actopaxin (LF-Actopaxin) in PLC, but not its corresponding short form readily enhanced cell migration in wound healing assays. Elevated protein levels of ILK, PINCH and phospho-paxillin, which are involved in focal adhesion complex formation and integrin signalling pathway, were also observed in LF-Actopaxin transfectants. Morphological changes were also observed in PLC LF-Actopaxin transfectants, showing enhanced stress fibre formation, filopodia and lamellipodia (cell protrusions) on the cell surfaces.

Conclusion: This study demonstrated for the first time the pro-migratory effects of Actopaxin in human HCC, and the existence of a short form which lacks a complete CH domain that is critical for cell migration, re-organisation of cytoskeletal events and turnover of focal adhesions. The differential effects of LF- and SF-Actopaxin on ILK, PINCH and phospho-paxillin protein levels might partly explain their influence on cell migration capacity in HCC cells. Further studies are warranted to investigate the potential role of Actopaxin in HCC tumour progression and cell invasioned stress fibre formation, filopodia and lamellipodia (cell protrusions) on the cell surfaces.

Study of arsenic trioxide sensitivity in human leukaemia: role of aquaporin 9 and its transcriptional regulators

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Introduction: Arsenic trioxide (As_2O_3) is an active drug in the treatment of acute promyelocytic leukaemia, and shows much promise in other haematological malignancies. It has previously been demonstrated that the transmembrane protein aquaporin 9 (AQP9) controls cellular arsenic uptake in myeloid and lymphoid leukaemia cells, and thus controls arsenic sensitivity. Consequently, leukaemia cells with higher AQP9 expression are more sensitive to As_2O_3. The objective of this study was to investigate the potential of drug-induced AQP9 upregulation in enhancing the sensitivity to As_2O_3-mediated cytotoxicity.

Methods and Results: Leukaemia cells were treated with the glucocorticoid dexamethasone (Dex) and the demethylating agent 5-aza-2′-deoxycytidine (DAC) separately. Increased AQP9 expression level and enhanced As_2O_3-mediated cytotoxicity after Dex or DAC treatment were demonstrated by quantitative PCR and MTT assay respectively. Treatment with glucocorticoid receptor antagonist RU486 reversed the effects of Dex, suggesting that Dex acted on the transcription of AQP9 through the glucocorticoid receptor. DAC treatment enhanced AQP9 expression in cell lines not expressing AQP9. Since analysis of the promoter region of AQP9 did not reveal any CpG islands, direct demethylation of AQP9 promoter is unlikely and demethylation of other transcription factor genes, which activate AQP9 expression might be involved. Several potential transcription factors were examined. Methylation studies showed that HNF1A was methylated in leukaemia cells. HNF1A expression was detected after DAC treatment and HNF1A siRNA abrogated the DAC-mediated AQP9 level upregulation. These results suggested that DAC increased the expression of AQP9 indirectly via demethylation and activation of HNF1A.

Conclusion: Dexamethasone and 5-aza-2′-deoxycytidine enhance As_2O_3-mediated cytotoxicity through upregulation of AQP9. Synergistic action between these drugs and As_2O_3 has potential therapeutic implication in the treatment of cancers that are resistant to As_2O_3 alone.

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Relationship of plasma interleukin-6 and its genetic variants with hypertension in Hong Kong Chinese

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Introduction: Interleukin-6 (IL-6) is a pro-inflammatory cytokine, which plays a central role in insulin resistance, atherogenesis, and hepatic production of acute phase proteins, such as C-reactive protein and fibrinogen. We investigated the relationship between plasma IL-6, its genetics variants and hypertension.

Methods: Four tagging single nucleotide polymorphisms (SNPs) were genotyped in 1936 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study–2 with a median follow-up of 6.4 years. Plasma IL-6 was measured using a high-sensitivity enzyme-linked immunosorbent assay (ELISA) in 942 subjects at follow-up.

Results: After genotyping, the SNP rs2069852 showed significant deviation from the Hardy-Weinberg equilibrium ($P<0.001$) and was excluded from all subsequent analysis. In stepwise multiple linear regression, the SNP rs1800796 was independently associated with plasma IL-6 level (beta= –0.098, P=0.002). High IL-6 levels were observed in subjects with hypertension (P=0.024), obesity (P=0.023), and the metabolic syndrome (P=0.009) after adjusting for age and sex. However, IL-6 was only independently associated with hypertension in women (P=0.002) after adjusting for other covariates. Although none of the SNPs showed significant association with prevalent hypertension, SNP rs2069837 was significantly associated with lower odds of incident hypertension (OR=0.66; 95% CI, 0.47-0.94; P=0.020). The two other SNPs, rs17147230 (OR=0.69; 95% CI, 0.48-0.99, P=0.044) and rs1800796 (OR=0.63; 95% CI, 0.39-1.00, P=0.048) also showed nominal association with lower odds of incident hypertension in women, but not men.

Conclusion: The inflammation marker, IL-6, appears to play an important role in hypertension, especially in women, in our population.

Acknowledgement: This study was funded by Hong Kong Research Grant Council grants (HKU7229/01M and HKU7626/07M), and the Sun Chieh Yeh Heart Foundation.

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Relationship of genetic variants in gene encoding adrenomedullin with hypertension and dysglycaemia in Hong Kong Chinese

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Introduction: Adrenomedullin (AM) is a vasodilatory peptide. It facilitates adipocyte differentiation and affects lipolysis and glucose uptake. Therefore, we investigated the association of common genetic variants in the adrenomedullin gene (*ADM*) with elevated blood pressure and dysglycaemia in the Hong Kong Chinese population.

Methods: Four single nucleotide polymorphisms (SNPs) in the *ADM* gene were genotyped in 1936 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study–2, which has a median follow-up of 6.4 years. Elevated blood pressure was defined as blood pressure ≥130/85 mm Hg or taking drug treatment. Dysglycaemia included impaired fasting glucose (≥5.6 mmol/L), impaired glucose tolerance (2h glucose ≥7.8 mmol/L) or diabetes.

Results: The minor T allele of SNP rs4910118 was significantly associated with lower systolic blood pressure (β= –0.057, P=0.0079) at baseline, but not at follow-up. However, none of the SNPs was significantly associated with prevalent or incident hypertension or elevated blood pressure. Although none of the SNPs was significantly associated with prevalent dysglycaemia at baseline, the SNP rs11042725 was significantly associated with prevalent dysglycaemia at follow-up (OR=1.24; 95% CI, 1.06-1.46; P=0.0093). Among subjects without dysglycaemia at baseline, the SNP rs11042725 was a significant predictor of incident dysglycaemia in women at follow-up (hazard ratio=1.46; 95% CI, 1.15-1.86; P=0.002), but not in men, using forward stepwise Cox regression analysis.

Conclusion: Our study suggests a stronger role of genetic variants in the *ADM* gene in the development of dysglycaemia than that of hypertension in our local Chinese population. Further studies on the genetic association of AM with dysglycaemia are warranted.

Acknowledgements: This study was funded by Hong Kong Research Grant Council grants (HKU7229/01M and HKU7626/07M), and the Sun Chieh Yeh Heart Foundation.

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Association of a genetic variant in the adiponectin gene with persistent hypertension in Hong Kong Chinese

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Introduction: We have previously found that low plasma adiponectin level was predictive of the development of hypertension in our local Chinese population. In this study, we investigated the associations of genetic variants in the adiponectin gene with plasma adiponectin level and hypertension.

Methods: A total of 14 single nucleotide polymorphisms (SNPs) were genotyped by the MassARRAY system (Sequenom, San Diego, CA) in 1936 subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study–2 (CRISPS-2). Plasma adiponectin level was measured by an in-house sandwich enzyme-linked immunosorbent assay in 1650 subjects.

Results: Plasma adiponectin level was significantly associated with four SNPs, rs12495941 (beta=0.100, P<0.0001), rs182052 (beta= –0.095, P<0.0001), -10677C>T (beta=0.067, P=0.0017), and rs266729 (beta= –0.071, P=0.0008). These SNPs were not associated with prevalent or incident hypertension among all the 1936 subjects. However, the SNP rs266729 was significantly associated with hypertension (odds ratio=1.49; 95% CI, 1.13-1.95; P=0.0044) and diastolic blood pressure (beta=0.113, P=0.018) in a sub-cohort of 1616 subjects who were normotensive or hypertensive for the whole 6.4-year follow-up period. In this sub-cohort, this SNP (odds ratio=1.39, P=0.020) was independently associated with hypertension in stepwise logistic regression. No significant sex interaction was found for the SNP with adiponectin level and hypertension.

Conclusion: Several genetic variants in the adiponectin gene influenced plasma adiponectin levels in our population and the SNP 266729 was associated with persistent hypertension in this population. Further studies on the role of genetic variants in hypertension are warranted.

Acknowledgements: This study was funded by Hong Kong Research Grant Council grants (HKU7229/01M and HKU7626/07M), and the Sun Chieh Yeh Heart Foundation.

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Association of a genetic polymorphism in the gene encoding fibrinogen beta chain with hypertension in Hong Kong Chinese

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Introduction: Elevated plasma fibrinogen level is associated with cardiovascular diseases. Two prospective studies in Caucasian populations showed positive association of plasma fibrinogen level with incident hypertension in men, but not in women. Single nucleotide polymorphisms (SNPs) in the gene encoding the fibrinogen beta chain (*FGB*) have been reported to be associated with plasma fibrinogen level. We therefore investigated the association of genetic variants in the *FGB* gene with hypertension.

Methods: Three tagging SNPs were genotyped in subjects from the Hong Kong Cardiovascular Risk Factor Prevalence Study cohort with a median follow-up period of 6.4 years. Genotyping was performed by the MassARRAY system (Sequenom, San Diego, CA) in 1294 subjects who had plasma fibrinogen level measured.

Results: The SNP rs4220 showed significant association with plasma fibrinogen level (beta=0.144, P<0.001 at baseline and beta=0.130, P<0.001 at follow-up). This SNP was also significantly associated with hypertension (odds ratio=1.49, P=0.004 at baseline and odds ratio=1.32, P=0.013 at follow-up). Among subjects without hypertension at baseline, this SNP was associated with incident hypertension in men (odds ratio=1.52, P=0.023), but not in women. This SNP showed a marginal non-significant sex interaction for incident hypertension (P=0.076).

Conclusion: As SNP rs4220 in the *FGB* gene is associated with both fibrinogen level and hypertension, fibrinogen may play a causal role in the development of hypertension development, especially in men.

Acknowledgements: The Hong Kong Cardiovascular Risk Factor Prevalence Study–2 was funded by Hong Kong Research Grant Council grants (HKU7229/01M and HKU7626/07M), and the Sun Chieh Yeh Heart Foundation. The genotyping of SNPs in the *FGB* gene was supported by a grant from the National Natural Science Foundation of China/Research Grants Council of Hong Kong Joint Research Scheme (30518001/CO301070202 and HKU720/05).

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Knockdown of survivin gene inhibits tumour growth and enhances chemosensitivity in hepatocellular carcinoma

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Introduction: Survivin is a member of the inhibitor of apoptosis family that plays a critical role in the control of cell division and inhibition of apoptosis. Expression of survivin is found only in transformed cell lines and human tumour tissues, such as colorectal cancer, but not in terminally differentiated adult tissue. In hepatocellular carcinoma (HCC), survivin mRNA is frequently expressed and its protein expression has been shown to be highly correlated to the proliferation index (Ki-67) rather than the apoptotic index.

Methods: This study aimed to investigate the functional effects of survivin on the tumourigenicity and chemosensitivity in HCC via the establishment of an HCC cell line (PLC/PRF/5) with the stably knockdown of survivin gene (PLC-k3).

Results: PLC/PRF/5 cells with stable suppression of survivin expression showed significantly lower survival rate and proliferation rate in cell viability and proliferation assays while comparing with the control cell line (PLC-v) harbouring the vector clone. In addition, higher chemosensitivity was observed in PLC-k3 cells in the above assays. Cell cycle study by flow cytometry showed that knockdown of survivin alone could induce cell cycle arrest, and lead to S phase arrest after cisplatin treatment. Different expression patterns of cell cycle proteins in PLC-v and PLC-k3 confirmed the above findings.

Conclusion: Results from in-vivo study supported our findings in in-vitro experiments that knockdown of survivin inhibited tumour growth and development in HCC. Our findings suggest that survivin plays a critical role in promoting cell proliferation rather than inhibition of apoptosis, and enhances chemosensitivity in HCC. Thus, the suppression of survivin expression in combination with cisplatin may contribute to the development of effective treatment in HCC.

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Circulating CD26+ cancer stem cells as a novel predictive marker for recurrence and development of metastasis in colorectal cancer

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Introduction: Recent studies have provoked the use of circulating tumour cells (CTCs) as a prognostic marker for disease progression in colorectal cancer (CRC), but its implication in disease prognosis or recurrence has been controversial. Based on our recent findings that CD26+ cancer stem cells (CSCs) can initiate distant metastases in CRC, we aimed to study the correlation of CD26+ CSCs in the patient tumour and circulation. The potential use of CTCs and circulating CD26+ CSCs as predictive markers of recurrence or distant metastasis in patients with primary or CRC liver metastases was also investigated.

Methods: Fifty-three patients undergoing resection of CRC primary and/or liver metastases were prospectively studied (36 primary CRCs, 4 synchronous CRC primary and liver metastases, and 13 metachronous CRC liver metastases). Blood samples were taken before surgery (T0), 1 month after tumour resection (T1), and thereafter every 3 months with a median follow-up of 2 years. In 28 of the patients, surgical specimens were also collected. The number of circulating and tumour tissue CSCs before and after surgery was analysed by multi-colour flow cytometry. CTCs were identified as being CD45-/CK8/18+/epCAM+, and circulating CSCs were identified as CD45-/CK8/18+/epCAM+CD26+. The level of circulating CD26+ CSCs was correlated with CD26+ CSCs in the resected tumours. The levels of CTCs, circulating CSCs and carcinoembryonic antigen (CEA) were correlated with tumour stage, recurrence and development metastasis by sensitivity and specificity tests.

Results: Our data demonstrated that the level of tumour CD26+ cells significantly correlated with circulating CD26+ cells in primary and metastatic CRC patients. Patients with metastases had higher levels of CTCs and circulating CSCs than those without. Post-resection circulating CD26+ CSCs at T1 decreased significantly in all patients. Moreover, patients with ≥0.05% preoperative circulating CD26+ CSCs developed metastases within 2 years, whereas none with ≤0.05% developed recurrences or metastases ($P<0.001$). For analysis of CTCs, only three of eight primary CRC patients with >0.03% CTCs developed recurrences or metastases, and only six of 13 metastatic CRC patients had >0.03% CTCs. Sensitivity and specificity of circulating CSCs ≥0.05% and CTCs ≥0.03% in predicting recurrences and metastases were 95% and 51%, respectively, and CEA level did not predict the development of recurrences or metastases.

Conclusions: Circulating CD26+ cell is a marker predictive of recurrence or distant metastasis that can be monitored non-invasively and should be further exploited for its potential use in the clinical setting.

Brain injury and neurogenesis after intracerebral haemorrhage in hypertensive rats

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Introduction: Effective treatments for intracerebral haemorrhage (ICH) are awaited to reduce its morbidity and mortality. Hypertension is the most important risk factor for ICH. Neurogenesis following ICH in nomotensive rats has been confirmed. In this study, we used a rat renovascular hypertension (RVHT) model, and investigated the effects of hypertension on the pathophysiological and histological changes, and neural stem cell proliferation after induction of ICH.

Methods: RVHT was achieved by applying a silver clip onto the left renal artery. At 6 weeks after renal artery constriction, the mRNA levels of angiogensin II type 1 (AT_1) and type 2 (AT_2) receptors in the brain were determined by reverse transcription–polymerase chain reaction. ICH was induced by an intrastriatal injection of bacterial collagenase IV in the left brain in both normotensive and hypertensive rats. Left femoral artery was cannulated for continuous monitoring of blood pressure for 4 hours after the induction of ICH. Haematoma volume was quantified at 24 hours after ICH induction. 5′-Bromo-2′-deoxyuridine (BrdU) was used to label cell proliferation from the 6th day to the 9th day after ICH. Rats were killed at 10 days after ICH. $BrdU^+$ and CD31 (an endothelial cell marker) immunoreactive cells were detected using immunofluorescence. Behavioural tests were performed at 1, 3, 7, 10, and 21 days after ICH.

Results: RVHT rats showed up-regulation of AT_1 receptor in the brain. Following induction of ICH, both the normotensive and RVHT rats demonstrated an acute hypertensive response. As compared to normotensive rats, RVHT rats demonstrated a larger haematoma volume, and greater deficits at all time-points. However, at 10 days after ICH, more $BrdU^+$ cells were detected over the perihaematoma area of RVHT rats than normotensive rats. Moreover, many $BrdU^+$ cells within the ipsilateral basal ganglia of RVHT rats also co-expressed CD31.

Conclusion: Renovascular hypertension aggravates histological and functional injury partly via up-regulation of AT_1 receptor in the brain. Increased brain injury in hypertensive rats induces increased neural stem cell proliferation and angiogenesis after ICH. Experimental ICH in hypertensive rats is a suitable model for evaluation of pathophysiology and treatment of patients with hypertensive ICH.

Acknowledgement: This research was supported by CRCG grants (200707176018 & 200807176052), The University of Hong Kong.

Non-invasive cryolipolysis for body contouring in Chinese patients—the first clinical experience

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Background: The objective of this study was to determine the patient satisfaction and clinical efficacy of a novel cryolipolysis device (Zeltiq) for body contouring in Chinese patients.

Methods: Twenty-two patients with 'discrete bulges' were recruited for this procedure. All patients received one single treatment using the Zeltiq Breeze System using treatment parameters of CIF 41.6 (-73 milliwatts/cm^2) for 60 minutes at the desired anatomical region. The areas treated were flank, back, and abdomen. At baseline visit, their weight was measured and caliper measurement was taken at the maximum area of fat when standing. Standardised 3D photographs were taken with the Vectra Canfield System. They were followed up 2 months after the treatment and were assessed by the physician. Thereafter, they had their weight, subcutaneous fat measured by caliper and photographs taken. Subjective assessments were evaluated by means of a questionnaire. Any adverse effects were documented.

Results: The preliminary data generated by 21 follow-ups all reported that the treatment was tolerable. Objective assessment by caliper showed a statistically significant improvement as compared with control (P=0.001). Physician assessment showed good to very good improvement in 17 (81%) out of 21 subjects. Thirteen of them thought the treatment length was just right and eight thought it was too long. 70% of the subjects felt satisfied to very satisfied. Of 21 subjects, 17 (81%) reported noticeable difference in the area treated. Eighteen of them would recommend the treatment to family and friends, while three of them were unsure.

Conclusion: For patients desiring a localised fat layer reduction, cryolipolysis offers a non-invasive, no-downtime procedure with high patient satisfaction and clinical efficacy.

Expression of aldehyde dehydrogenase isoforms in acute myeloid leukaemia

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Introduction: Aldehyde dehydrogenase (ALDH) is a family of enzymes comprising 19 isoforms in human which are involved in the oxidation of both endogenous and exogenous aldehydes to the corresponding acids. ALDH activity is consistently associated with normal haematopoietic stem cells activity in mice and human and more recently leukaemia initiating activity and inferior prognosis in acute myeloid leukaemia (AML). The specific isoforms associated with the reported ALDH activity are unclear. To address this, we examined the expression of ALDH isoforms in bone marrow (BM) samples from healthy donors as well as AML patients.

Methods: Seven ALDH genes (1A1, 1A2, 1A3, 1B1, 3A1, 5A1, and 8A1)—of which 3A1 and 5A1 are involved in cyclophosphamide metabolism, 1B1 was reported to be a possible marker in granulopoiesis, and 1A1, 1A2, 1A3 and 8A1 are involved in endogenous retinoic acid biosynthesis—were examined. Purified $CD34^+$ or mononuclear cells from six normal and nine AML BM were investigated. Three AML cell lines were also studied. Reverse transcription–polymerase chain reaction was performed to amplify a specific segment of different ALDH isoforms.

Results: Among the ALDH tested, ALDH1A3 was expressed in all six normal but not in any AML BM samples or cell lines. ALDH3A1, ALDH5A1 and ALDH8A1 were not expressed in normal BM. However, they were expressed in 7/10, 8/10 and 5/10 AML samples and 1/3, 3/3 and 2/3 cell lines respectively. No differential gene expression between normal and AML samples were observed in other ALDH isoforms.

Conclusion: Specific ALDH isoforms exhibited differential gene expression between normal and AML BM. Their roles in normal and neoplastic haematopoiesis should be further evaluated.

Acknowledgements: The project was supported by the General Research Fund (HKU 7520/06 M and HKU 770308 M) and a grant from the strategy research theme of cancer stem cells in the University of Hong Kong.

Diffuse large B-cell lymphoma of the central nervous system in mycophenolate mofetil–treated patients with systemic lupus erythematosus

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We report the third case of primary lymphoma of the central nervous system (PCNSL) in a patient with systemic lupus erythematosus (SLE) given long-term mycophenolate mofetil (MMF). Our 43-year-old patient has a history of lupus nephritis and has been treated with MMF 500 mg/day in addition to azathioprine (AZA) for 8 years. She presented with subacute left-sided weakness. Magnetic resonance imaging revealed a gadolinium-enhancing mass in the right parietal region which was isointense on T2-weighted imaging. Brain biopsy revealed diffuse sheets of large lymphoid cells which demonstrated strong membranous expression of CD20 by immunohistochemistry and positive signal for Epstein Bar virus (EBV)–encoded RNA by in-situ hybridisation study. Complete remission of PCNSL was achieved after discontinuation of MMF and administration of rituximab and whole brain radiotherapy.

Patients with SLE are predisposed to development of lymphoma regardless of immunosuppressive use. One meta-analysis found that non-Hodgkin's lymphoma was more common in SLE patients with a standardised incidence rate ranging from 5.2 to 44.4. However, the development of PCNSL secondary to immunosuppressive use is being increasingly recognised especially in MMF-treated renal transplant recipients with onset of PCNSL after a median of 14 months. It has also been described in some MMF-treated autoimmune conditions such as myasthenia gravis, dermatomyositis and relapsing polychondritis. Although AZA in combination with corticosteroids has been shown to predispose post-renal transplant patients to lymphoproliferative disease with a relative risk of 12.7, the association of AZA and EBV-related lymphoma is rare. The approach to management of this condition includes withdrawal of MMF and judicious use of future immunosuppressive agents.

Clinical outcome of relapsing remitting multiple sclerosis among Hong Kong Chinese

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Background: Many relapsing remitting multiple sclerosis (RRMS) patients develop irreversible progressive neurological disability. Reported clinical outcome varied. We aimed to study clinical outcome of Chinese RRMS patients.

Methods: Only RRMS patients with MRI brain and/or spinal cord abnormalities fulfilling Barkhof's criteria for dissemination in time and space followed up in our hospital were recruited for this retrospective study. Patients with neuromyelitis optica or neuromyelitis optica spectrum disorders were excluded.

Results: Eighty RRMS patients were studied. Their mean onset age was 27.5 (range, 12-50) years, mean disease duration was 16.8 (range, 1.5-30) years; 61 (73%) were female. Seventy-two (90%) patients had CSF oligoclonal bands; 74 (93%) patients had spinal cord MRI abnormalities compatible with inflammatory demyelination. Their mean number of relapses in the first 2 years was 1.8 (range, 0-6). At latest follow-up, 24% patients had EDSS score 2 or less, 33% had EDSS 2.5-4, 54% had EDSS 4 or more, 36% had EDSS 6 or more, and 41% patients developed secondary progressive multiple sclerosis. The median time from symptom onset to reach EDSS 6 was 22 years, and the median age at reaching EDSS 6 was 59 years old. Multivariate cox-regression analysis revealed that demographic characteristics, presenting neurological features, number of relapses in the first 2 years and immunomodulatory therapies (azathioprine and beta-interferon) did not affect time to reach EDSS 6.

Conclusion: The median time for our relapsing remitting multiple sclerosis patients from symptom onset to reach EDSS score 6 (walking require unilateral assistance) was 22 years. Beta-interferon did not confer long-term neurological benefit in RRMS.

Expression of Sox7 in lymophoid leukaemia cells and umbilical cord blood

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Introduction: Sox proteins are a family of transcription factors with high-mobility-group DNA-binding domain (HMG box) homologous to SRY, which are implicated in embryogenesis and neoplastic transformation. However, their roles in leukaemogenesis are unclear. Human Sox7, constituting a subfamily with Sox17 and Sox18 were cloned and characterised. In this study, we examined the expression of Sox7 in leukaemia, especially in lymphoid leukaemia cells and umbilical cord blood (UCB) cells.

Methods: Bone marrow (BM) or peripheral blood samples of patients with myeloid (acute myeloid leukaemia [AML], myelodysplastic syndrome [MDS], chronic myelogenous leukaemia [CML]) and acute lymphoid leukaemia (ALL) and samples of UCB were prospectively collected. Mononuclear cells were isolated by Ficoll. CD34+, CD34- subfractions and CD34+CD38+ and CD34+CD38- subfractions were isolated by microbeads or by sorting. Expression of Sox7 was evaluated by reverse transcriptase–polymerase chain reaction (RT-PCR) and quantitative real-time PCR (Q-PCR). Methylation of CpG island in Sox7 promoter was evaluated by methylation-specific PCR.

Results: Sox7 was relatively highly expressed in ALL and normal samples while exhibited almost undetectable expression in myeloid leukaemia cells. In cord blood, all of the six samples showed Sox7 expression. Sox7 expression in CD34+ subfraction is higher than that in CD34- cells of ALL samples and UCB. Sox7 expression in CD34+CD38+ is lower than that in CD34+CD38- subfraction of ALL samples and UCB. In the 12 patients selected, Sox7 promoter of the patient without Sox7 expression is methylated. The other samples showed Sox7 expression and no methylation was observed in the respective Sox7 promoter.

Conclusion: Sox7 gene is expressed in most cases of ALL and normal BM and UCB. However, it was silenced in myeloid malignancies including AML, MDS and CML. In ALL and UCB, it was preferentially expressed in the primitive CD34+CD38- population, suggesting its role in the regulation of haematopoietic and leukaemia stem cells.

Acknowledgements: The project was supported by the General Research Fund (HKU 7520/06 M and HKU770308M) and a grant from the strategic Research Theme of cancer stem cells in the University of Hong Kong.

The relationship of asthma and the pattern of adiposity in adult Chinese

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Introduction: While epidemiological studies of asthma using body mass index (BMI) support an association between asthma and obesity, little is known about the pattern of adiposity associated with asthma. Using different markers for obesity, we compared the characteristics of adiposity in Chinese adult asthmatics with non-asthmatics in a match-controlled study.

Methods: A total of 399 Chinese patients, age ≥35-74, with persistent asthma and requiring long-term inhaled corticosteroids, from the territory of Hong Kong were matched 1:1 on age, gender, and BMI with controls from the Hong Kong Cardiovascular Risk Factors Prevalence Study II from a random population sample. The independent association of asthma with waist circumference (WC), waist-hip-ratio (WHR), and central obesity (defined as WC ≥90 cm in men and 80 cm in women) was evaluated with multivariate regression adjusting for age, gender, BMI, education, smoking, alcohol consumption, physical activity, and the total numbers of prescriptions of oral steroids.

Results: The mean age was 53.5±10.5 years with 42% males in both groups, mean BMI was 23.66±3.72 kg/m^2 in asthmatics compared with 23.68±3.56 kg/m^2 in controls (P=0.94). Asthmatics had a significantly larger WC (82.09 ±10.25 cm vs 78.85±9.46 cm; P<0.001) and higher WHR (0.86±0.07 vs 0.84±0.07; P<0.001), even after adjustment for confounders including BMI compared with controls. Moreover, central obesity was significantly more prevalent in asthmatics versus controls (adjusted OR=3.59; 95% CI, 2.20-5.86).

Conclusion: Asthma is significantly associated with central obesity. The effect of central obesity on asthma control, and adverse health influences of central obesity in patients with asthma need to be further explored.

Acknowledgement: This research was supported by Small Project Fund, The University of Hong Kong.

Quantification of *Pneumocystis jirovecii* in patients with or without *Pneumocystis jiroveci* pneumonia

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Objectives: *Pneumocystis jirovecii* pneumonia (PCP) is potentially fatal in immunocompromised patients. We developed a real-time quantitative polymerase chain reaction (Q-PCR) targeting the mitochondrial large subunit rRNA (*mtLSUrRNA*) gene to understand the infection by this organism.

Methods: A total of 261 broncho-alveolar lavage (BAL) from 47 immunocompetent and 213 immunocompromised patients with or without PCP were examined by silver-stain and Q-PCR.

Results: Silver-stain in 47 specimens revealed eight positive samples (from seven immunocompromised patients). Q-PCR in all specimens revealed 17 positive samples (from 16 patients), including all eight silver-stain–positive cases, four silver-stain–negative cases, and five cases that had not undergone microscopic examination. Silver-stain–positive samples contained more *mtLSUrRNA* copies/ng DNA than negative ones (median: 1.61x10^2, 6.40x10^0-44x10^6 vs median: 0, 0-3.38x10^5; P<0.01). The sensitivity and specificity of Q-PCR for *P jirovecii* were 100% and 89% respectively. The positive and negative predictive values for positive silver-stain were 68% and 100% respectively. Of 178 BAL samples from immunocompetent patients without pneumonia, four (2.0%) were Q-PCR positive. None developed PCP, qualifying as cases of colonisation.

Conclusions: Q-PCR was a sensitive and specific diagnostic test for *P jirovecii*. Colonisation of the lower respiratory tract in immunocompetent patients was uncommon.

Primary care service utilisation rates and pattern of the Hong Kong population

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Background: Primary health care is generally accepted as a major care provider, and but there were little data on the utilisation of primary health care services in Hong Kong.

Methods: A cross-sectional study of 5174 telephone contacts with 3148 subjects (response rate, 60.8%) completing a structured questionnaire on primary health care service utilisation rates and pattern and socio-demographics was conducted. The rates of illness and service use in the last 4 weeks, pattern of utilisation during last illness and last consultation were measured. The rates and pattern of utilisation of people with and without a regular family doctor was compared.

Results: The mean population number of consultations in 4 weeks was 0.7 per person. The proportion of people who had visited western medicine practitioner, Chinese medicine practitioner and A&E department, prevalence rate in last 4 weeks were 24.6%, 9.5% and 3.7%, respectively. A total of 71.7% had consulted a doctor in the last episode of illness including 65.4% consulting western medicine practitioners, 12.1% Chinese medical practitioners, 7.3% attended A&E department and 3.1% were admitted to the hospital. Over half of last consultations were private western medicine (64.2%) including 59.8% general practices and 4.4% in specialists, whereas over one fifth (23%) of patients visited government-funded general out-patient clinics (15.6%) and specialists (7.4%). Respectively only 8.2% and 2.7% patients had utilised Chinese medicine and other health services at their last consultation. People with a regular family doctor were less likely to use A&E department or hospital service in the last 4 weeks (2.7% vs 5.0%), and less likely to use public services in last consultation (11.1% vs 30.9%).

Conclusion: The primary care service utilisation rates in Hong Kong are high compared with countries where primary care is provided by a more unified system. The family doctor–led primary care may reduce the use of A&E department and hospital services.

Acknowledgement: This research was supported by Studies in Health Services Grant (No. SHS-P-10) from the Food and Health Bureau of the Hong Kong Special Administrative Region.

1α, 25-dihydroxyvitamin D3 suppresses differentiation, maturation and activation of dendritic cells from patients with systemic lupus erythematosus

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Background: Dendritic cells (DC), professional antigen presenting cells, are believed to play a crucial role in the pathogenesis of systemic lupus erythematosus (SLE). 1α, 25-dihydroxyvitamin D3 (VitD3), in addition to its effect on bone metabolism, has been increasingly recognised to have immunomodulatory effects.

Objective: To examine the effect of VitD3 on the differentiation, maturation, and activation of DCs in SLE patients.

Methods: $CD14^+$ monocyte–derived DCs from SLE patients and age- and sex-matched controls were derived from growth medium cultured with IL-4, GM-CSF. Mature DCs were induced by addition of lipopolysaccharide and tumour necrosis factor-α in the presence or absence of VitD3 (1×10^{-10} M) and/or dexamethasone (1×10^{-6} M). The expression of CD1a, a DC marker and markers of maturation and co-stimulatory molecules such as CD80, CD86, CD40, HLA-DR and CD83 were examined by flow cytometry. After stimulation of DCs with CD40L for 24 hours, the production of pro-inflammatory cytokines including IL-12 and IL-6, were measured by ELISA kits.

Results: VitD3 suppresses differentiation of monocytes into DCs as showed by the decreased expression of CD1a ($P<0.05$). VitD3 inhibits the expression of maturation markers including CD86, CD40 and CD83 ($P<0.05$), but not CD80 and HLA-DR. This effect was more marked in SLE patients (n=14) than controls (n=9). In combination with dexamethasone, VitD3 displayed more potent immunosuppressive effect on DCs. Under the effect of VitD3, stimulated DCs produced less of IL-12 (3.1 vs 10.4 pg/mL, P=0.02) and IL-6 (216.0 vs 224.0 pg/mL, P=0.21) in SLE patients as well as controls (8.0 vs 36.6 μg/mL, P=0.01 for IL-12) and (380.7 vs 415.2 pg/mL, P=0.04 for IL-6).

Conclusion: VitD3 is found to inhibit differentiation, maturation, and activation of DCs in vitro in both SLE patients and controls and may be considered as immunomodulatory agent in the treatment of SLE.

Beta1 subunit–dependent modulation of BK channel by membrane cholesterol

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Background: The large conductance Ca^{2+}-activated K^+ (BK or Maxi-K) channels are ubiquitously expressed in different tissues without (brain, liver, etc) or with (smooth muscle and heart) regulatory beta-subunit, and play an important role in regulating various physiological processes such as cell excitability, hormone secretion, vascular activity, etc. Recent studies have shown that membrane cholesterol is a major regulator of several potassium channels including Kir and Kv1.5 channels. However, the regulation of BK channels by cholesterol is not fully understood.

Methods: Whole cell BK current and BK single channel current were recorded in whole-cell patch clamp mode and cell-attached single channel recording, respectively, in HEK 293 cells stably expressing Maxi-K gene or Maxi-K with beta1-subunit.

Results: We found that whole-cell BK current was significantly suppressed with cholesterol depletion by methyl-beta-cyclodextrin (MCD), whereas cholesterol-saturated–MCD had no effect on the current amplitude. Single channel recording showed that cholesterol depletion significantly reduced the open probability of BK channel, suggesting that cholesterol depletion likely decreases the membrane channel number. Interestingly, in cells stably expressing Slo and beta1-subunit, depletion of membrane cholesterol increased BK current, while cholesterol-saturated–MCD reduced BK current.

Conclusion: Our results demonstrate the important evidence that BK channels exhibit beta1-subunit–dependent responses to cholesterol. The enriched cholesterol reduces the activity of BK channels co-expressed with Max-K and beta1-subunit, which may at least in part accounts for the occurrence of hypertension in patients with high plasma cholesterol level, since beta-subunit transcripts are abundant in vascular smooth muscle.

Phase I/II trial of PTK787/ZK222584 combined with intravenous doxorubicin for treatment of patients with advanced hepatocellular carcinoma: implication for anti-angiogenic approach to hepatocellular carcinoma

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Background: This is a phase I/II trial aiming to assess the efficacy and tolerability of PTK787/ZK222584 (PTK) in combination with intravenous doxorubicin for the treatment of advanced hepatocellular carcinoma (HCC) patients.

Methods: In phase I, advanced HCC patients received PTK at escalating doses together with doxorubicin 60 mg/m^2 given as an intravenous bolus every 3 weeks to establish the maximum tolerated dose (MTD). Subsequently, in phase II, all patients received the same regimen with oral PTK at the MTD dose together with doxorubicin 60 mg/m^2 given as an intravenous bolus every 3 weeks for a maximum of 6 cycles.

Results: Nine patients were recruited in phase 1 part with the MTD established as 750 mg daily. Overall, 27 patients received the regimen with PTK at 750 mg daily. The median age was 52 (range, 23-73) years and 63% of patients were chronic hepatitis B carriers. Notably, the majority of patients had Child B cirrhosis. The overall response rate was 26.0% with all patients having partial response, and another 20% of patients achieved stable disease for at least 12 weeks. The median progression-free survival was 5.4 (0.27-23.6) months and overall survival was 7.3 (0.8-23.6) months. The commonest grade 3 or 4 non-haematological toxicities were mucositis (11%) and alopecia (7%), respectively. Grade 3 or 4 neutropenia was observed in seven (26%) patients with two having neutropenic sepsis.

Conclusion: The combination of PTK with intravenous doxorubicin shows synergistic activity in advanced HCC patients. Thus, the idea of combining anti-angiogenic agent together with chemotherapy may warrant further evaluation in future clinical trials of advanced HCC patients.

A phase I study of pazopanib in patients with advanced hepatocellular carcinoma

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Background: Patients (pts) with advanced hepatocellular carcinoma (HCC) have a poor prognosis. HCC is a highly vascular tumour with increased levels of angiogenic factors including VEGF and VEGFR. Pazopanib (GW786034) is an oral angiogenesis inhibitor targeting VEGFR, PDGFR, and c-Kit. A phase I study was conducted to determine the maximum tolerated dose (MTD), safety, pharmacokinetics, pharmacodynamics and efficacy of pazopanib in patients with locally unresectable and/or advanced HCC.

Methods: Eligibility criteria included unresectable and/or metastatic HCC with at least one target lesion, recovery from prior systemic regimens, ECOG PS 0 or 1, Child-Pugh A, and adequate organ function. Doses of pazopanib were escalated from 200 mg once daily (QD) to 800 mg QD in a 3 + 3 design. DCE-MRI was performed to assess changes in tumour permeability.

Results: Twenty-seven Asian pts have been enrolled at QD doses of 200 (4 pts), 400 (10), 600 (8), 800 (5)—median (range) age, 61 (38-76) years; M/F=85%/15%; ECOG 0/1=59%/41%; 81% with metastatic disease; 67% with stage IV; 22% with prior systemic therapy, 26% with prior TACE. Most common AEs were: diarrhoea (59%; 4% Gr 3); hypertension (44%; 26% Gr 3), cough (19%); fatigue (19%; 4% Gr 3); and hair depigmentation (15%). Hepatobiliary lab abnormalities were: AST elevation (63%; 15% Gr 3), hyperbilirubinemia (63%; 4% Gr 3), ALT elevation (41%; 7% Gr 3), and Alk phos (37%; 4% Gr 3). DLTs were Gr 3 malaise (1 pt) and Gr 3 AST/ALT elevation (1 pt) at 800 mg. MTD was determined to be 600 mg QD. Median (range) days on study was 85 (4-663) overall; 106 days (4-274) at the MTD. Best response was PR in 2 pts (7%; 1 at 800 mg, 1 at 600 mg) and SD >4 mos in 11 pts (41%). Median TTP at the MTD was 137.5 (4-280) days. Median % change in tumour permeability (K^{trans}) following 3 weeks of pazopanib administration at the MTD (5 pts) was 45%. Predose and maximum plasma pazopanib concentrations at 800 mg QD were similar to values observed previously at the same dose.

Conclusions: Pazopanib has a manageable safety profile in HCC at the MTD of 600 mg QD. Preliminary evidence of antitumour activity was observed. Changes in tumour DCE-MRI parameters were seen following repeated dose pazopanib administration.

Transient carcinoembryonic antigen elevations during adjuvant chemotherapy for colorectal cancer reflect the burden of residual micrometastatic disease

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Background: Carcinoembryonic antigen (CEA) testing is routinely used to monitor progress of advanced cancer patients on treatment, or else to detect relapse during follow-up, particularly in colorectal cancer (CrC). Although CEA levels have been reported to rise during adjuvant drug therapies, the mechanism of such 'surges' is not clear. This study was conducted to clarify the clinical significance of this phenomenon.

Methods: We conducted a retrospective analysis of CEA levels in 88 consecutive patients receiving adjuvant chemotherapy in our centre—39 patients with primary CrC, and a comparison cohort of 49 adjuvant breast cancer patients (BrC). In the event of two serial CEA rises, endoscopic and/or imaging investigations were performed to exclude recurrence. Subset analyses were based on nodal status and primary tumour type.

Results: Primary resection was associated with significant CEA decline in CrC but not BrC patients. Forty-three (48.9%) patients experienced CEA fluctuations exceeding 0.5 ng/mL during adjuvant chemotherapy; CEA rises indicated true recurrence in two (4.7%) patients. Adjuvant CEA surges occurred both more often and more extensively in disease associated with ≥4 positive nodes in CrC but not in BrC patients ($P<0.05$).

Conclusion: Both the frequency and extent of CEA surges during adjuvant chemotherapy parallel the severity of preoperative nodal involvement in CrC, but not in BrC, suggesting that such surges reflect tumourilytic effects on occult disease in CrC patients only. Whether these CEA surges predict survival that is inferior (ie due to greater burden of residual disease) or superior (ie due to greater tumourilytic efficacy) to that of stage-matched 'non-surge' patients, however, remains to be determined by larger prospective CrC studies.

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Drop in alpha-foetal protein level in the first 6 weeks is an early surrogate for survival benefit in patients on sorafenib for advanced hepatocellular carcinoma

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Introduction: The role of serum alpha-fetoprotein (AFP) changes in predicting the response and survival benefit of advanced hepatocellular carcinoma (HCC) patients to sorafenib remains unknown.

Methods: A retrospective study was performed to analyse the early changes of AFP level in advanced HCC patients who enrolled in our phase II open-label sorafenib study. Serum AFP was collected prospectively at baseline and during each follow-up visits in parallel with radiological and survival outcomes. AFP responders were defined as those patients who had relative drop of AFP >20% of the baseline level at 6 weeks of sorafenib treatment. The relationship between AFP response and the radiological disease control (DC) rate was examined and the relationship of AFP drop to survival outcomes was also evaluated.

Results: Of 51 patients enrolled in a phase II study of sorafenib monotherapy for advanced HCC, 41 had elevated baseline AFP level (>20 ng/mL). Nine patients were AFP responders, whereas 32 patients were AFP non-responders. Overall, eight patients had radiological DC by sorafenib. AFP response (P=0.04) was significantly associated with radiological DC and the relative chance of radiological DC for AFP responders versus non-responders was estimated to be 3.4 (95% CI, 1.1-11.1). Multivariate analysis indicated that AFP response was associated with significant better progression-free survival (hazard ratio=0.31; 95% CI, 0.13-0.76; P=0.01) and marginally with better overall survival (hazard ratio=0.30; 95% CI, 0.09-1.02; P=0.05).

Conclusion: Drop in AFP level in first 6 weeks may be an early surrogate for response and survival benefits in patients receiving sorafenib for advanced HCC with elevated baseline AFP level.

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Phase II trial of sorafenib with capecitabine and oxaliplatin (SECOX) in patients with locally advanced or metastatic hepatocellular carcinoma

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Background: This is a single arm, multi-centre, phase II study to assess the efficacy and tolerability of sorafenib combining oxaliplatin and capecitabine (SECOX) for the treatment of advanced hepatocellular carcinoma (HCC) patients.

Methods: Advanced HCC patients with no prior systemic therapy received SECOX regimen—sorafenib 400 mg twice a day (day 1-14), oxaliplatin 85 mg/m^2 (day 1) and capecitabine 1700 mg/m^2 (day 1-7) every 2 weeks. Response assessment using RECIST criteria was performed after 4 cycles. Patients who achieved partial response or stable disease would receive another 4 cycles till a maximum of 8 cycles. Afterwards, sorafenib was continued till disease progression. The primary endpoint was time-to-progression (TTP) and the secondary endpoints were tumour response rate (RR), overall survival (OS) and tolerability.

Results: A total of 51 patients were enrolled in the trial. The median age was 58 (range, 28-81) years and all patients were in ECOG performance status 0-1. Eighty-four percent of patients were chronic hepatitis B carriers and 98% of patients had Child A cirrhosis. Ten (20%) patients had tumour vascular invasion and 41 (80%) patients had extra-hepatic metastasis. The best RR was 14% and another 61% of patients achieved stable disease. Overall, 75% of patients derived clinical benefits from SECOX regimen for at least 8 weeks. The median TTP was 7.1 (1.7-19.9) months and OS was 10.2 (2.1-20.5) months. Hand-foot-skin reaction (73%), diarrhoea (69%), and neutropenia (63%) were the most commonly encountered toxicities, with the majority of patients having grade 1 or 2 toxicity. No treatment-related death was reported.

Conclusion: The SECOX regimen demonstrates highly significant clinical activity and good tolerability in advanced HCC patients. Our data support a randomised trial comparing SECOX versus sorafenib alone for treatment of advanced HCC.

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Identification of miR-21 and miR-106b overexpression in hepatoceullar carcinoma by an orthotopic metastasis mouse model

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Introduction: Hepatocellular carcinoma (HCC) is an aggressive tumour with dismal prognosis. The poor prognosis of HCC is mainly due to the development of distant metastasis. Recent evidence suggests that deregulation of microRNAs (miRNAs) contributes to tumourigenesis and may constitute robust biomarkers for cancer diagnosis and prognosis. Understanding the molecular mechanisms leading to the development of extra-hepatic metastasis may shed new light on the clinical and functional implications of miRNA deregulation in this aggressive malignancy.

Methods: In this study, we aimed to identify specific miRNAs that are involved in the development of metastasis by means of an in-vivo orthotopic tumour metastasis model. Using two sets of primary liver tumour cell lines and their corresponding lung metastasis counterparts, cells were orthotopically injected into the liver of SCID mice and observed for development of metastasis. Metastases developed from the cell lines were subjected to miRNA microarray analysis to identify differentially expressed miRNAs that are associated with the development of metastasis in vivo. In-vitro assays were also performed to demonstrate the functional significance of ectopic down-regulation of miR-21 and miR-106b. The metastatic capacity of cells with stable miR-21 and miR-106b suppression was also investigated by in-vivo orthotopic models.

Results: Fifteen human miRNAs, including miR-21 and miR-106b, were differentially expressed in two metastatic cell lines compared with the primary tumour cell lines. In addition, the clinical relevance of miR-21 and miR-106b was further demonstrated by expressional studies in 99 HCC paired tumourous samples to confirm its role in HCC carcinogenesis. The results demonstrated that both miR-21 and miR-106b were over-expressed in HCC tumour tissues when compared with the adjacent non-tumour controls. Knockdown of miR-21 and miR-106b expression led to enhanced invasiveness and migratory capacity of HCC cells in vitro, and efficiently suppressed the development of distant metatasis in vivo.

Conclusion: Our findings suggested that miR-21 and miR-106b may be involved in the development of hepatocarcinogenesis. In addition, their role in the development of distant metastasis is also established. Further functional studies on the molecular mechanisms of both miR-106b and miR-21 are warranted.

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Toll-like receptor 4 deficiency alleviate hepatic inflammation in non-alcoholic steatohepatitis in ApoE knockout Mice

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Background: Hepatic inflammation is the major feature of non-alcoholic steatohepatitis (NASH). Toll-like receptor 4 (TLR4) is the important mediator of the systemic inflammatory responses. The molecular link between hepatic inflammation and the pathogenesis of NASH remains elusive. The present study aimed to investigate the role of TLR4 in hepatic inflammation in NASH.

Methods: ApoE-/-/TLR4-/- double knockout (DKO) mice were generated by mating the ApoE KO mice with TLR4 KO mice. DKO mice and their TLR4 wild-type (littermates) were fed with high-fat high-cholesterol (HFHC) diet for 12 weeks to induce NASH. The extent of liver injury was assessed by histological examination in liver tissue and measurement of serum alanine aminotransferase (ALT) activity. Intrahepatic messenger RNA expression of cytokines, including tumour necrosis factor α (TNF-α), monocyte chemoattractant protein–1, and interleukin-6 were assessed by real-time PCR. Protein expression was determined by western blot analysis.

Results: HFHC diet induced steatohepatitis in ApoE KO mice, as indicated by steatosis in combination with the increased infiltration of inflammatory cells in the liver tissue, elevated serum ALT levels, and up-regulated expression of TNF-α. By contrast, serum ALT levels were significantly suppressed in DKO group in comparison to that in TLR4 wild-type group. DKO mice had a reduction of macrophage infiltration as demonstrated by the down-regulation of F4/80 and CD68, two well-established markers for macrophages. In addition, the expression of TNF-α was decreased in DKO mice compared to that in TLR4 wild-type group.

Conclusions: TLR4 deficiency alleviates hepatic inflammation induced by HFHC diet. Thus, blocking the hepatic inflammation by TLR4 mutation is sufficient to suppress the progression from simple steatosis to steatohepatitis.

Relationship of systemic inflammation to arterial stiffness in patients with psoriasis

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Background: Psoriasis is associated with increased cardiovascular risk, which is not explained by traditional cardiovascular risk factor but may be in part due to increased arterial stiffness, an independent predictor of cardiovascular mortality. In the present study, our aim was to establish whether psoriatic patients have increased arterial stiffness and to investigate the relationship between inflammation and arterial stiffness.

Methods: A total of 47 psoriasis patients and 54 normal subjects were enrolled into the study. Baseline demographics, blood tests including high-sensitivity C-reactive protein (hsCRP) and psoriatic disease activity including Psoriasis Area and Severity Index (PASI) and Nail Psoriasis Severity Index (NAPSI) were studied. Arterial stiffness was assessed by heart-ankle pulse wave velocity (haPWV).

Results: The male sex (72.3 vs 79%, P=0.48) and mean age (43.1±7.7 vs 44.7±8.4 years) were similar between psoriatic patients and control subjects. Psoriatic patients had higher hsCRP levels (4.52±4.37 vs 2.58±2.54 mg/L, P=0.02) and haPWV (8.14±2.54 vs 7.14±0.72 ms^{-1}, P=0.02) compared to control subjects. In psoriatic patients, haPWV (r=0.52, P<0.01), PASI (r=0.52, P<0.01) and NAPSI (r=0.37, P=0.04) were positively correlated with hsCRP. Furthermore, stepwise multiple regression analyses demonstrated haPWV correlates independently with hsCRP (R^2=0.46, P=0.01).

Conclusion: Psoriasis is associated with increased arterial stiffness, which correlates with active inflammation measured by hsCRP. Effective control of inflammation may be of benefit in reducing cardiovascular risk in patients with psoriasis.

High plasma osteoprotegerin is associated with impaired vascular functions in type 2 diabetes

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Introduction: Recent studies showed that plasma osteoprotegerin, a new marker of atherosclerosis, is elevated in type 2 diabetic patients with coronary artery disease even in the absence of clinical atherosclerosis. In addition, plasma osteoprotegerin correlates with coronary artery calcification and aortic plaque formation, and predicts cardiovascular disease and vascular mortality over a 10-year period. We investigated the relationship between plasma osteoprotegerin and vascular function in Chinese patients with type 2 diabetes and no clinical manifestation of atherosclerosis.

Methods: Sixty-four Chinese type 2 diabetic patients (age, 56.2±9.9 years; male, 56.3%) treated with diet alone, or metformin and/or sulfonylurea for at least 6 months, were studied. Anthropometric parameters, blood pressure, fasting lipids, fasting glucose/insulin, high-sensitivity C-reactive protein (hsCRP) and various pro-inflammatory proteins were measured. Osteoprotegerin was measured using ELISA assay. Endothelial function of the brachial artery was assessed by measuring flow-mediated dilatation (FMD) and nitroglycerin-mediated dilatation (NMD) using high-resolution vascular ultrasound.

Results: Plasma osteoprotegerin correlated with age (r=0.67, P<0.001), systolic blood pressure (r=0.26, P=0.037), and soluble tumour necrosis factor–alpha receptor II (r=0.35, P=0.005). There were no correlations between plasma osteoprotegerin and diastolic blood pressure, interleukin-6, and hsCRP. FMD and NMD of the brachial artery were negatively correlated with plasma osteoprotegerin level (r= –0.27, P=0.034 and r= –0.31, P=0.012 respectively), while carotid intima media thickness (cIMT) was positively correlated (r=0.25, P=0.044). Linear regression analysis showed that osteoprotegerin was an independent determinant of FMD (P=0.027) and NMD (P=0.012). Other independent determinants of FMD and NMD were hsCRP and body mass index. Age, male gender, and systolic blood pressure, but not osteoprotegerin (P=0.056) were the independent determinants of cIMT.

Conclusion: Our study has demonstrated the independent association of high plasma osteoprotegerin levels with impaired vascular functions. Whether plasma osteoprotegerin is a marker of the underlying inflammatory process or a direct participant in the development of atherosclerosis requires further studies.

Rapamycin reduces interleukin-6–induced inflammatory mediators in human mesangial cells

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Introduction: Rapamycin is an immunosuppressive agent with proven efficacy in the prevention of kidney transplant rejection. Its role in immune-mediated glomerular diseases remains to be investigated. Interleukin-6 (IL-6) is a pro-inflammatory cytokine involved in the pathogenesis of various mesangial proliferative glomerulonephritides. We investigated the effect of exogenous IL-6 and rapamycin on cell activation, signalling pathways and chemokine secretion in human mesangial cells (HMC).

Methods: Confluent growth arrested HMC were stimulated with IL-6 (10 ng/mL) for durations of up to 72 hours in the presence or absence of rapamycin (3 ng/mL). The culture media was decanted and cells lysed with 4 M urea buffer.

Results: IL-6 induced activation of mTOR, ERK and phosphatidylinositol-3-kinase, accompanied by increased alpha-smooth muscle actin and MCP-1 secretion after 24-72 h stimulation ($P<0.05$ for all, compared to serum-free medium). Rapamycin significantly inhibited mTOR and ERK activation, but had no effect on phosphatidylinositol-3-kinase activation induced by IL-6. Rapamycin also reduced MCP-1 secretion and mesangial cell activation that were induced by IL-6 ($P<0.05$ for all, with vs without rapamycin), with a maximum inhibitory effect observed after 72 hours of incubation.

Conclusion: Our data demonstrated that rapamycin could ameliorate inflammatory processes induced by IL-6 in HMC, and thus may have a therapeutic role in mesangial proliferative glomerulonephritides.

Acknowledgements: This study was supported by a RGC GRF grant (7550/06M), UGC Matching Grant Scheme (Phase II), Endowment Fund established for the 'Yu Professorship in Nephrology' awarded to Professor TM Chan, and the Wai Hung Charitable Foundation Limited.

Effects of mycophenolic acid and cyclophosphamide on inflammatory processes in human mesangial cells

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Introduction: Mycophenolate mofetil (MMF) or cyclophospamide (CTX), combined with corticosteroid, is the conventional treatment for severe lupus nephritis. There are increasing data that mycophenolic acid (MPA), the active metabolite of MMF, can exert a direct effect on cells other than lymphocytes. It is not known whether CTX has direct effects on resident renal cells. We have previously reported that human polyclonal anti-dsDNA antibodies (HPAb) induced cytokines and matrix proteins in human mesangial cells (HMC). This study investigated the effect of MPA and CTX on inflammatory mediators induced by HPAb in HMC.

Methods: HPAb were isolated from the sera of 12 patients with lupus nephritis using affinity chromatography. Confluent growth arrested HMC were incubated with HPAb or control IgG (10 μg/mL) for up to 72 hours.

Results: HPAb induced IL-6 secretion, NFkappa-beta activation and apoptosis (TUNEL-positive cells) in HMC in a time-dependent manner after 24-72h incubation ($P<0.05$ for all, compared to control IgG). MPA (5 μg/mL) abrogated HPAb-induced NFkappa-beta activation, with decreased IL-6 secretion and apoptosis ($P<0.05$ for all, with vs without MPA). In comparison, CTX (10 μg/mL) had no significant effect on cytokine release, activation of NFkappa-beta, or apoptosis in HMC.

Conclusion: These data demonstrated the direct but differential effects of MPA and CTX on changes in HMC function induced by HPAb, with potential therapeutic implications.

Acknowledgements: This study was supported by a RGC GRF grant (7366/04M), UGC Matching Grant Scheme (Phase II), Endowment Fund established for the 'Yu Professorship in Nephrology' awarded to Professor TM Chan, and the Wai Hung Charitable Foundation Limited.

Four-and-a-half LIM protein 2 promotes invasive potential and epithelial-mesenchymal transition in colon cancer

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Introduction: Cancer invasion and metastasis may be associated with the phenotype transition called epithelial-mesenchymal transition (EMT). As an adaptor for protein interactions, the four-and-a-half LIM protein 2 (FHL2) has oncogenic potential in gastrointestinal cancers. The aim of this study was to evaluate the role of FHL2 on EMT and invasion of colon cancer.

Methods: FHL2 over-expression in stable transfectants or suppression by siRNA was used. Expression of vimentin, MMP9 and E-cadherin was detected by RT-PCR, real-time PCR and western blot. The trans-activity of beta-catenin was determined by luciferase-reporter system and the detection of its downstream genes. The composition of E-cadherin/beta-catenin complex was visualised under fluorescence microscopy.

Results: FHL2 was overexpressed in colon cancer penetrating through basement membrane. FHL2 siRNA inhibited while FHL2 over-expression promoted invasion capacity of cancer cells. FHL2 expression was inducible by TGF-beta and FHL2 mediated TGF-beta induced vimentin expression. Over-expression of FHL2 increased while FHL2 siRNA suppressed the expressions of vimentin and MMP-9. Furthermore, FHL2 siRNA suppressed the trans-activity of beta-catenin and inhibited the expressions of its downstream genes survivin and cyclin D1 through modulating the phosphorylation of beta-catenin. FHL2 siRNA increased E-cadherin expression and the presentation of membrane-associated E-cadherin/beta-catenin complexes.

Conclusion: We conclude that FHL2 promotes EMT of colon cancer through modulating the organisation of E-cadherin/beta-catenin complex and may facilitate the invasion or metastasis of colon cancer.

Human cardiac Kv4.3 channels are regulated by protein tyrosine kinases

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Introduction: The transient outward K^+ current I_{to} (encoded by Kv4.3) plays an important role in the phase 1 rapid repolarisation of cardiac action potentials in the heart. Modulation of I_{to} by intracellular signal transduction is largely unknown.

Methods: The present study was designed to determine whether hKv4.3 channel (α-subunit of human cardiac I_{to}) is regulated by protein tyrosine kinases (PTKs) in HEK 293 cells stably expressing human Kv4.3 gene using a whole-cell patch-clamp technique, immunoprecipitation and western blot.

Results: It was found that human cardiac Kv4.3 current amplitude was remarkably inhibited by the broad-spectrum PTK inhibitor genistein (10 μM), and the inhibition was partially antagonised by the protein tyrosine phosphoatases inhibitor orthovandate (1 mM). It is interesting that the selective EGFR (epidermal growth factor receptor) kinase inhibitor AG556 (10 μM) reversibly reduced Kv4.3 current, and the inhibitory effect was almost fully countered by orthovandate. In addition, the Src-family kinase inhibitor PP2 (10 μM) also decreased hKv4.3 current and the effect was partially antagonised by orthoavanadate. Immunoprecipitation and western blot analysis revealed that tyrosine phosphorylation level of hKv4.3 channel was reduced by genistein, AG556 or PP2. Their reduction of hKv4.3 channel phosphorylation level was reversed by orthovanadate.

Conclusion: These results demonstrate that hKv4.3 channel is regulated by both EGFR kinase and Src-family kinases. EGFR and Src-family kinases favour tyrosine phosphorylation of the channel, and therefore may affect the cardiac repolarisation.

Regulation of cell proliferation by ion channels in human mesenchymal stem cells

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Introduction: Human bone marrow–derived mesenchymal stem cells (hMSCs) are a promising cell source for regenerative medicine; however, cellular physiology is not fully understood in hMSCs. The present study was to determine the potential role of the dominant functional ion channels, large-conductance Ca^{2+}-activated K^+ (BKCa) channel, human ether-à-go-go K^+ (hEAG1) channel, and Na^+ channel, in regulating proliferation of hMSCs.

Methods: Ionic currents were recorded using a whole-cell patch-clamp technique. Cell proliferation assay was made with MTT and ^{3}H-thymidine incorporation approaches. Cell cycle distribution was determined by flow cytometry.

Results: We found that the BKCa channel blocker paxilline (1 μM) almost fully inhibited BKCa current (from 6.76±0.99 pA/pF of control, to 0.02±0.09 pA/pF at +100 mV, n=5, P<0.05) in hMSCs. The hEAG1 channel blocker astemizole (0.5 μM) significantly reduced hEAG1 current from 4.28±1.86 pA/pF to 1.40±1.13 pA/pF at +50 mV, n=6, P<0.05). The MTT experiment showed that paxilline at 0.3, 1.0, and 3.0 μM reduced cell proliferation to 97.2, 84.4, and 48.7% of control, respectively, and astemizole at 0.3, 0.5, and 1 μM decreased cell proliferation to 96.5, 80.5, and 45.8%, respectively. However, the Na^+ channel blocker tetrodotoxin (1 μM, fully blocked Na^+ current) had no effect on proliferation in hMSCs. Both paxilline and astemizole reduced DNA synthesis rate in a concentration-dependent manner. Inhibition of BKCa channel with 1 μM paxilline or hEAG1 channel with 0.5 μM astemizole accumulated cells at G0/G1 phase (from control 68.9% to 80.5% for paxilline; to 79.2% for astemizole).

Conclusion: Our results demonstrate that BKCa and hEAG1 channels, but not Na^+ channel, participate in the regulation of cell proliferation by promoting G0/G1 cells into cell cycling progression.

Human embryonic stem cells–derived mesenchymal stem cells functionally attenuate monocrotaline-induced pulmonary arterial hypertension in mice

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Introduction: Transplantation of bone marrow (BM)–derived mesenchymal stem cells (MSCs) has been shown to attenuate pulmonary arterial hypertension (PAH). However, the effect of human embryonic stem cells (hESCs)–derived MSC which may have higher proliferative capacity than BM-MSCs on the pulmonary vascular bed in monocrotaline (MCT)-induced animal model of PAH has not been determined. In the present study, the effects of hESC-MSCs versus BM-MSCs transplantation on MCT-induced pulmonary arterial hypertension (PAH) were compared in mice.

Methods: PAH was induced in adult mice (ICR strain) by intraperitoneal injection of 400 mg/kg MCT. As the negative control, mice received saline instead of MCT (control group, n=6). One week after MCT administration, the animals were randomised to receive intravenous administration of: (1) PBS (MCT group, n=6); (2) $3.0x10^6$ BM-MSCs (BMC group, n=6); or (3) $3.0x10^6$ hESC-MSCs (hESC group, n=6) via tail vein. All animals were treated with cyclosporine (15 mg/kg) daily after transplantation. Invasive haemodynamic assessment and immunohistological studies were performed at 3 weeks after transplantation.

Results: Administration of either hESC-MSCs or BM-MSCs significantly attenuated elevated RV systolic pressure and reduced RV hypertrophy. After 1 week of transplantation, both hESC-MSCs and BM-MSCs not only retained in the wall of pulmonary vessels and in lung parenchyma, but also underwent vascular differentiation and cytokine release. However, after 3 weeks of transplantation, both BM-MSCs and hES-MSCs were undetectable in lung tissues as confirmed by immunostaining for human nuclear antigen (HNA) and PCR. Both hESC-MSCs and BM-MSCs were able to reduce microvascular wall thickness and increase density of pulmonary capillary to augment MCT-induced PAH.

Conclusion: We conclude hESC-MSCs are as functional as BM-MSCs to attenuate MCT-induced PAH. Despite hESC-MSCs have a higher proliferative capacity, both cell types are poor to long-term survive well in injured lung environments.

In-vitro combination of arsenic trioxide and chemotherapy in small-cell lung cancer

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Introduction: Arsenic trioxide (ATO), an anti-tumour agent with multi-faceted mechanisms of action, has become a breakthrough treatment for acute promyelocytic leukaemia in recent years. There have been preliminary data about the potential activity of ATO in solid tumours, including small-cell lung cancer (SCLC). As SCLC is considered a chemo-sensitive malignancy, we conducted an in-vitro study examining the cytotoxic effects of ATO, clinically effective chemotherapeutic agents, or a combination of both in a SCLC cell line model.

Methods: Drug treatment (ATO, cisplatin, etoposide) experiments were performed in 4 SCLC cell lines (H-187, DMS-79, H-526, H-69) obtained from ATCC. The cancer cell viability was assessed by 3-(4,5-dimethyl-thiazoyl-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The proportion of apoptotic cell death was detected by Annexin-V/Propidium iodide assay with flow cytometry. The effects of combination treatment were determined by isobologram analysis using standard computer software (CalcuSyn, Biosoft, US).

Results: All the SCLC cell lines reacted to ATO and traditional chemotherapeutic reagents in time- and dose-dependent way. Two of them (H-187, H-526) were sensitive to ATO (eg IC50[48 hr]=2.4±0.35 μM and IC50[48hr]=2±0.21 μM), while the other two SCLC cell lines, DMS-79 and H-69, were resistant to either ATO (eg IC50[48hr]=12.3±1.5 μM and 10±3.7 μM) or chemotherapeutic agents (IC50=10-50 μM). Moderate synergistic cytotoxicity or additive effect were found in ATO and cisplatin combination treatment either in sensitive or resistant cell lines (CI=0.5-0.9). On the other hand, antagonistic interaction was shown in the combination of ATO and etoposide in all 4 cell lines (CI= 0.9-2).

Conclusion: Combination of ATO and cisplatin was synergistic in chemo-sensitive and additive in chemo-resistant SCLC models. However, the combination of ATO and etoposide has resulted in antagonism. Further study is needed to determine the possibility and best schedule of combination treatment of ATO with existing standard chemotherapy.

'Yin-Yang' regulation of insulin signalling by APPL1 and APPL2 in skeletal muscle cells

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Introduction: APPL1 and APPL2 are two intracellular adapter proteins containing a PH domain, a PTB domain, and a Leucine zipper motif. A growing body of evidence suggests that APPL1 acts as a key signalling molecule integrating multiple signalling stimuli. We have recently demonstrated that APPL1 potentiates insulin actions in liver and adipose tissue. However, the physiological functions and the underlying molecular mechanisms of APPL1 and APPL2 in regulating insulin actions in skeletal muscle have not been explored. The objective of this study was to investigate the role of APPLs in insulin signalling and downstream glucose uptake in both cultured myocytes and rodent models.

Methods: Proteins physically associated with APPL1 or APPL2 were retained by affinity purification and co-immunoprecipitation, followed by mass spectrometry–based proteomic identification. The key domains of APPL2 involved in its interaction with APPL1 and TBC1D1 were determined by progressive truncation and site directed mutagenesis. Effects of APPL1 and APPL2 in regulating insulin signalling were measured by Akt phosphorylation and in-vitro or ex-vivo glucose uptake assay.

Results: Overexpression of APPL2 inhibits insulin-stimulated Akt phosphorylation leading to down-regulation of glucose uptake in C_2C_{12} myotubes and in skeletal muscle of the APPL2 transgenic mice. In contrast, suppressing APPL2 expression by RNAi significantly enhances insulin-stimulated Akt phosphorylation and glucose uptake in C_2C_{12} myotubes. However, APPL1 exerts opposite effects in regulating insulin signalling in muscles compared to APPL2. Co-immunoprecipitation assay followed by western blot analysis revealed the interaction of TBC1D1 with APPL2 but not APPL1. Furthermore, the interaction occurs in the N-terminal PTB domain of TBC1D1 and N-terminal BAR domain of APPL2. The binding of APPL2 to TBC1D1 was enhanced by insulin-stimulated Akt activation, and suppressed by overexpression of APPL1.

Conclusion: APPL1 and APPL2 act as a pair of 'Yin-and-Yang' molecules critically involved in the regulation of insulin signalling and glucose uptake in skeletal muscle. Further investigations on these two proteins might lead to the identification of novel regulatory mechanisms that underlie insulin resistance and diabetes.

XIAP-associated factor 1 (XAF1), a novel target of p53, enhances p53-mediated apoptosis via a post-translational modification mechanism

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Background: X chromosome–linked inhibitor of apoptosis protein (XIAP)–associated factor 1 (XAF1) acts as a negative regulator of the IAP family members to mediate apoptosis. The present study aimed to determine the putative interaction and mechanism between XAF1 and p53 in human gastric and colon cancer cells, especially during process of apoptosis.

Methods: Using chromatin immunoprecipitation (CHIP) assay to investigate the interaction between p53 protein and XAF1 promoter. The XAF1 and p53 expressions were detected by reverse transcription–polymerase chain reaction (PCR) and western blot analysis. Luciferase reporter assays were used to detect activities of various promoter of XAF1 and p53 transcriptional regulation. UV exposure and adriamycin (ADR) were used to induce DNA damage on stable cell line AGS with high expression of XAF1.

Results: We first showed that XAF1 is a novel target gene of p53. Wild type but not mutant p53 was able to downregulate XAF1 at both mRNA and protein levels, which initiated from physical interaction with XAF1 promoter. In turn, over-expression of XAF1 was capable of activating p53 via post-translational modification in response to DNA damage, thereby resulting in enhancing p53 nuclear accumulation and transcriptional activity. In addition, XAF1 enhanced p53-dependent apoptosis.

Conclusions: These results suggest that XAF1 is a novel target gene of p53. Moreover, XAF1 can activate p53-mediated apoptosis via enhancing the post-translational modification of p53.

Krit1 inhibited proliferation and metastasis of human colon cancer via DPPIV signalling pathway

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Background: Loss of function of Krev interaction trapped-1 (Krit1) mutations contributed to cerebral cavernous malformation-1 (CCM1). Krit1 may help determine cell shape and adhesion, both crucial in angiogenesis. The aim of this study was to elucidate the potential mechanism of Krit1-midiated cell proliferation and metastasis in colon cancers.

Methods: Human colon cancer cell line HCT116 was stably transfected with full-length Krit1. Cell proliferation and invasion were measured by colony formation assay, invasion assay, and wound healing assay. Both xenograph nude mice tumour model and orthotopic nude mice tumour model were used to investigate the effects of Krit1 overexpression on tumour growth and metastasis.

Results: Overexpression of Krit1 in HCT116 cells resulted in down-regulation of colony formation ($P<0.01$) and inhibition of wound recovery and invasion. Stable expression of Krit1 significantly decreased tumour volume ($P<0.05$) and the incidence of liver metastasis in vivo. Our studies showed that overexpression of Krit1 led to restoration of dipeptidyl peptides IV (DPPIV) expression, which in association with decreased expression of downstream chemokine stromal–derived factor1 (SDF1) and its receptor CXCR4. DPPIV inhibitor Diprotin A treatment resulted in restoration of cell proliferation and migration potential in Krit1 stable expressed cells.

Conclusions: Our results showed that in colon cancer Krit1 inhibited cell proliferation and invasiveness by upregulation of DPPIV signalling pathway.