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Plenary 1

Autoimmune encephalopathies caused by synaptic antibodies

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Synaptic autoimmunity is mediated by immunoglobulins that target plasma membrane receptor and channel proteins effecting cell to cell communication in the nervous system. This concept was first hypothesised as the basis of the postsynaptic neuromuscular transmission defect in myasthenia gravis.[1] In 1971 the concept was extended to the autonomic and central nervous systems and to plasma membrane receptors on endocrine cellular targets of disorders that associate with myasthenia gravis (eg, thyroid epithelium, gastric parietal cells, pancreatic beta-islet cells, and ovarian follicular cells.[2] Autoantibody effectors of synaptic autoimmunity are usually of IgG class. They bind to the ectodomain of target channels or neurotransmitter or hormone receptors with high affinity and impair their function directly (by activating, blocking or preventing allosteric transition) or indirectly (by inducing endocytosis and degradation secondary to antigen cross-linking, or via concomitant endocytosis of a physically coupled non-antigenic protein [eg, an excitatory amino acid transporter] or via inflammatory/cytotoxic sequelae of complement activation). IgGs targeting synaptic autoantigens are not found in healthy subjects.

Synaptic autoimmune disorders involving the central nervous system are recognised increasingly today in both idiopathic and paraneoplastic contexts. Symptom onset may be subacute or insidious. The presentation may be diverse: psychosis, dementia, movement disorder, sensory, motor, seizure, dyssomnia, ataxia, nausea, vomiting, inappropriate anti-diuresis, coma, dysautonomia, or hypoventilation. Paraneoplastic cases reflect anti-tumour immune responses initiated by onconeural signalling proteins in cancers that are limited in spread and unsuspected at presentation (eg, small-cell lung carcinoma, ovarian teratoma, seminoma, thymoma or lymphoma). Recognised neural targets include presynaptic, postsynaptic and perisynaptic elements (neuro-neuronal, neuro-endocrine, astro-neuronal, astro-oligodendroglial and astro-endothelial synapses). Identified antigens include neuronal voltage-gated potassium channels (and their interacting synaptic and axonal proteins, LGI1 and CASPR2) and calcium channels (P/Q-type and N-type), neuronal nicotinic acetylcholine receptors, ionotropic glutamate (NMDA and AMPA) and glycine receptors, metabotropic GABA-B, GLU-R1 and GLU-R5 receptors and the astrocytic aquaporin-4 water channel. Most of these disorders generally respond favorably to early-initiated antibody-depleting therapies. Long-term immunosuppressant therapy (at least 5 years) may be required to maintain remission. The search for neoplasia is aided by the individual patient's extended autoantibody profile. If an associated tumour is found, its resection or ablation may improve the neurological outcome.

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Genomics of Taiwan young-onset hypertension study

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Hypertension is a common and complex disorder with great public health impact. To identify genetic variants may contribute to genetic screening and management of hypertension and in turn reduce burden of cardiovascular diseases. Although many large-scale genome-wide association studies have been performed, very few studies have successfully identified replicable large-impact hypertension loci, not to mention the scanty Asian studies. Young-onset hypertension (YOH) is considered as a more promising target disorder to investigate than the late-onset one due to its stronger genetic component.

Through two large series of YOH studies (a family-based and a case-control study), we have performed linkage analysis and genome-wide association study employing various types of tests and methods such as single locus, multilocus, gene-based tests, copy number variation, and pathway analysis. Gene-based and pathway-based approaches are more powerful than others probably due to locus heterogeneity nature of hypertension. In addition, gene mapping for endophenotypes of hypertension such as adiponectin and angiotensin-converting enzyme activity has been very successful and highly replicable. A summary of the findings from Taiwan YOH study will be presented.

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Monitoring disease activity of ankylosing spondylitis patients in a Chinese cohort: Bath Ankylosing Spondylitis Disease Activity Index or Ankylosing Spondylitis Disease Activity Score

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Background and Objectives: Recently, a new index, the Ankylosing Spondylitis Disease Activity Score (ASDAS), has been shown to be a validated and highly discriminatory instrument in assessing disease activity. The objective of this study was to evaluate the performance of ASDAS, and making comparison with the existing instrument, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), in a local Chinese ankylosing spondylitis (AS) cohort in a cross-sectional setting. In addition, the role of high-sensitivity C reactive protein (CRP) assays (hs-CRP) in AS assessment and ASDAS calculation was studied.

Methods: Data, including BASDAI, Bath Ankylosing Spondylitis Functional index (BASFI), Visual Analogue Scale (VAS) for spinal pain, patient and physician global assessments were gathered during clinic visits. Inflammatory markers, including erythrocyte sedimentation rate, CRP and hs-CRP were collected. ASDAS was calculated accordingly. The discriminatory capacity of BASDAI and ASDAS was compared by: (1) standardised mean difference statistics, (2) R-squared in linear regressions, and (3) area under receiver operating characteristics curve (AUC) in logistic regression models.

Results: ASDAS and BASDAI showed good correlation with patient and physician global assessment. Both indices demonstrated good discriminatory capacity, with high AUC in the logistic regression models (AUC >0.9). However, there was no significant difference in the performance between them. Overall, inflammatory markers correlate poorly with disease activity (r<0.3). Measurement of hs-CRP did not provide extra benefit.

Conclusions: ASDAS and BASDAI show similarly good performance. The role of hs-CRP in AS disease assessment is minimal.

A population-based study of 408 cases of acute promyelocytic leukaemia showing changes in epidemiology and prognosis during two decades: impact of oral arsenic trioxide

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Background: The incidence of acute promyelocytic leukaemia (APL) shows racial variations. Population-based epidemiologic studies are feasible because of common diagnostic criteria and treatment. The prognosis of APL has substantially improved since the advent of all-trans-retinoic acid (ATRA) and arsenic trioxide (As_2O_3).

Methods: Data on survival and relapse of consecutive APL patients in Hong Kong from 1991 to 2011 were obtained from the Hong Kong Cancer Registry (with at least 98% reporting and complete follow-up), and verified against hospital pathology and computer records. Data were censored at the end of July 2011. Potential factors impacting on survival (age; Plt; WCC [as continuous variables]; gender; year cohort and use of As_2O_3 maintenance) were studied by logistic regression analysis.

Results: A total of 408 cases of APL (198 men, 210 women) at a median age of 41 (3-89) years were registered. There was a rise in cases number with successive 5-year cohorts, but the WHO standardised age incidence rate (WSIR) was unchanged. The median haemoglobin level at diagnosis (Dx) was 8.4 (2.9–14.9) g/dL, WBC: 17.7 (0.3-250) x 10^9/L (>10 x 10^9/L in 129 cases) and Plt: 35 (3-270) x 10^9/L (<40 x 10^9/L in 282 cases). Early death (within 30 days of Dx) occurred in 88 cases. Complete remission (CR) was achieved in 318 cases. Outcome was unknown in 2 cases. The incidence of early death decrease with year cohort (P=0.035), and was positively related to older age (P<0.001), high WBC (P<0.001) and male gender (trend only, P=0.06). From CR1, the median follow-up was 83 (0-249) months. Relapse occurred in 108 cases. The 5-year relapse rate fell from 54% (no maintenance) to 16% (P<0.001) with the adoption of ATRA (n=110) and oral As_2O_3 (n=88; since 2001) maintenance. Relapse rates were lower in patients receiving As_2O_3 than ATRA maintenance (17% vs 38%; P=0.008). Risk factors predicting relapse were older age (P=0.001), no oral- As_2O_3 maintenance (P=0.003), high WBC (P=0.023), and male gender (trend only, P=0.056). For relapsed patients, 5-year overall survival (OS) from CR1 was improved from 67% with ATRA (1993-1998) to 96% with oral-As_2O_3 (since 1999-2011) treatment. The causes of deaths after CR1 were APL relapse (n=35), second cancer (n=8), bone marrow transplantation (BMT; n=7), chemotherapy (n=3), and unrelated causes (n=6). Allogeneic BMT was not performed since 1999. For the last 5-year cohort (2006-2011), there were no APL-related deaths after CR1. With reduction of induction death, and improvement in treatment and prevention of relapses, the 5-year OS from diagnosis increased from 44% for the first 5-year cohort to 80% for the last 5-year cohort. On multivariate analysis, age (P<0.001) and cohort period (P=0.031) were determinants of OS.

Conclusions: Population-based incidence data showed that APL in Hong Kong was more prevalent than the United States (0.15-0.18/100 000/year). Oral-As_2O_3 has markedly changed the outcome of APL patients. Early death is now the greatest problem curtailing survival of APL patients.

Preliminary experience of oral arsenic trioxide–based therapy in the treatment of refractory mantle cell lymphoma

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Background: Oral arsenic trioxide (As_2O_3, Arsenol™) is an active agent in acute promyelocytic leukaemia. It is licensed for the treatment of haematological malignancies in Hong Kong. It has been historically used for the treatment of low-grade lymphomas, which also shows in-vitro cytotoxic activity in mantle cell lymphoma (MCL) cell lines. We update our 7-year clinical experience of using oral As_2O_3 in refractory lymphoma patients.

Methods: A total of 38 relapse/refractory MCL were recruited. Daily oral As_2O_3 (10 mg), ascorbic acid (AA, 1 g) and chlorambucil (Clb, 2-4 mg) were given as out-patient until maximum response or the disease judged refractory. In responding patients, maintenance with arsenic and AA for 2 weeks was given every month for a planned 2 years, and further tailed off. Treatment was resumed at disease progression.

Results: There were 31 men and 7 women, with a median age of 65 (range, 43-90) years treated. The median time from initial MCL diagnosis to oral As_2O_3 treatment was 33 (range, 6-174) months. The median number of previous treatment was 2 (range, 1-7), and the median time from last lymphoma therapy to As_2O_3 treatment was 2 (range, 0 -31) months. A total of 26 patients (68%) showed documented response (10 CR/CRu, 16 PR). In 15 responding cases, Clb was stopped after a median of 4 (range, 2-15) months, and oral As_2O_3 monotherapy was continued for a median of 16 (range, 4-37 months). At a median follow-up of 13 (range, 0.7-87) months, a total of 21 patients died, mostly of progressive lymphoma. Two responding patients died of pneumonia. Disease progression occurred in 11 responders at a median of 17 (range, 2-60) months. At the last follow-up, a total of 17 patients were alive at a median of 24 (range, 0.7-87) months from initial arsenic salvage. Reversible liver enzyme derangement (n=10), herpes zoster reactivation (n=9), gastrointestinal discomfort (n=12) were the most common reported **side-effects. No prolonged QT interval or arrhythmia was recorded.**

Conclusion: Our results compare favourably with other novel salvage therapy for refractory MCL. A combination of oral As_2O_3, AA ± alkylators is a feasible out-patient–based option for relapsed and refractory MCL and can result in sustained disease control and amenable to maintenance treatment.

Acute renal failure in acute promyelocytic leukaemia: risk factors and outcome

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Background: Acute renal failure (ARF) requiring renal replacement therapy is a rare complication of acute promyelocytic leukaemia (APL). The underlying risk factors and mechanisms, and its relation to all-trans-retinoic acid (ATRA) and arsenic trioxide (As_2O_3) therapy are unknown.

Methods: The case history of all APL cases treated at Queen Mary Hospital either at initial presentation (n=29) or referred for oral As_2O_3 therapy either in remission (n=52) or relapse (n= 45) were reviewed. ARF was defined by the need for renal replacement therapy. In one patient, blood levels of elemental arsenic were measured by induction couple spectroscopy during the course of haemodialysis as replacement therapy.

Results: Among 126 consecutively treated APL patients, ARF occurred in 7 cases, 6 during initial diagnosis and 1 at first relapse. Leukocyte activation (pre-eclampsia, n=1; sepsis, n=5; acute arthritis, n=1; granulocyte-colony stimulation factor administration, n=1) was the common predisposing factor. ARF occurred both before (n=3) and after (n=4) ATRA administration, including one case treated with ATRA plus As_2O_3 at relapse. This patient did not have ARF during ATRA induction previously but suffered from ARF with perineal abscess during ATRA plus As_2O_3 treatment at relapse. Demographics and clinicopathologic characteristics were similar in patients with or without ARF. Three patients died. Four survivors achieved complete remission (CR), and remained in molecular remission. Two patients have completed oral arsenic maintenance therapy (creatinine [Cr], 168 and 100 μmol/L; normal range, 67-109 μmol/L). One patient had been on maintenance ATRA plus As_2O_3 for 18 months (Cr, 200 μmol/L). The final case in CR2 required long-term haemodialysis (Cr, 274-635 μmol/L) and had been on maintenance As_2O_3 for 6 months (1-2 mg single dose after haemodialysis session). The serum level of arsenic was steady in therapeutic range between dialysis sessions.

Conclusion: The prognosis of ARF in APL was unfavourable. As ARF is associated with either the APL differentiation syndrome or disorders leading to significant leukocyte activation, clinicians should be fully alert of the possibilities of renal dysfunction when these conditions arise. Aggressive treatment and early institution of corticosteroids may be needed in order to avert the occurrence of ARF. In patients on renal replacement therapy, oral As_2O_3 appears to be safe and effective, but its blood levels need close monitoring.

A retrospective analysis of 21 cases of drug hypersensitivity syndrome in a tertiary hospital

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Introduction: Drug hypersensitivity syndrome or drug reaction with eosinophilia and systemic symptoms (DRESS) is a severe idiosyncratic drug-induced reaction, carrying a mortality of 10%. Our aim was to investigate the demographic data, clinical, biochemical and histopathological characteristics, causative agents, treatment, and outcome of local cases of DRESS.

Methods: A retrospective study on 21 cases of DRESS from 2007 to June 2011 was carried out. Demographic data, pattern of causative drugs, clinical course, biochemical characteristics, histopathological findings, treatment, and outcome of all cases were analysed.

Results: Age of patients ranged from 14 to 77 years. Allopurinol was the most common causative drug, followed by aromatic anti-convulsants and cotrimoxazole. Fever, exanthematous rash and eosinophilia were consistently observed (100%). Lichenoid dermatitis was the commonest histopathological findings. Elevated liver parenchymal enzymes were the most common visceral involvement (91%). Allopurinol was associated with a higher incidence of renal involvement in DRESS than other drugs ($P<0.01$). Cotrimoxazole was associated with persistent erythroderma and might represent a long-term cutaneous complication of DRESS. The mortality rate was 5%.

Conclusion: Early diagnosis and prompt discontinuation of the offending agent are important in the management of DRESS. Aggressive treatment with corticosteroid is warranted in selected severe cases to minimise morbidity and mortality. Frequent and close monitoring is necessary due to a multitude of complications, high relapse rate and protracted disease course.

Dementia and all-cause mortality in Chinese nursing home residents: a 2-year prospective cohort study

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Background: The relationship between dementia and increased mortality in nursing home residents is controversial. Functional status is a potential confounding factor. The objective of this cohort study was to investigate the association of dementia with mortality with the influence of functional status.

Methodology: This was a 2-year prospective cohort study conducted from July 2009 to June 2011. Older Chinese nursing home residents from nine nursing homes in Hong Kong were included. Subjects were categorised by the presence and absence of dementia according to the DSM-IV criteria. Demographics, co-morbidity according to Charlson Co-morbidity Index (CCI) and Barthel Index (BI) for functional status were collected at baseline. Subjects were followed up for 2 years. All-cause mortality was recorded.

Results: A total of 812 older Chinese adults were recruited; 599 had dementia and 213 did not have dementia. Baseline characteristics were well matched between the two groups except the functional status. A higher proportion of low functional status was present in the dementia group than the non-demented group. The 2-year all-cause mortality rate of the dementia group was significantly higher than that of the non-demented group (38.6% vs 23.7%; $P<0.001$). After stratification, there were no significant difference in mortality between the dementia group and non-demented group in both the high (BI $\geq$60) and low (BI <60) functional status subgroup ($P=0.80$). Cox regression also showed the presence of dementia was not an independent predictor for all-cause mortality but functional status was an independent predictor.

Conclusion: In Chinese older nursing home residents, the dementia diagnosis is not related to increased mortality. The presence of co-existing low functional status is related to increased mortality in these residents. Functional assessment in older nursing home residents is therefore important to predict the prognosis.

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Efficacy of influenza vaccination in institutionalised older residents: importance of functional assessment

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Background: Overestimation of efficacy of influenza vaccination in older adults was frequently suggested due to frailty selection bias. There were little clinical data regarding the correlation between functional status of older adults and vaccine efficacy.

Methodology: From December 2009 to November 2010, a prospective cohort study about the efficacy of dual vaccination of monovalent (H1N1) 2009 and trivalent seasonal influenza on older residents of nine nursing homes was conducted. Subjects were divided into three groups; good (GF group: Barthel Index [BI], >60), intermediate (IF group: BI, 5 to 60), and poor (PF group: BI, 0) functional status group. Each subject consented to receive both trivalent seasonal influenza vaccination and monovalent (H1N1) 2009 vaccination (H1N1-TIV), or trivalent seasonal influenza vaccination alone (TIV only), or refused both vaccinations (unvaccinated). Outcome measures included all-cause mortality and mortality due to pneumonia based on ICD-9-CM.

Results: A total of 711 older residents were included; 230 were in the GF group, 246 in the IF group, and 235 in the PF group. There was no significant difference in baseline characteristics in the three groups. The 12-month all-cause mortality and mortality due to pneumonia were significantly higher in PF group and lower in GF group. Baseline functional status of subjects was an independent predictor for mortality. After multivariate analysis, the efficacies of H1N1-TIV versus TIV alone, H1N1-TIV versus unvaccinated, and TIV alone versus unvaccinated were all higher in GF group and lower in PF group.

Conclusion: Functional status of nursing home older residents was an important factor for determining the efficacy of influenza vaccine. To provide a better estimation of efficacy and minimise frailty selection bias, update functional status assessment by validated functional assessment instrument is essential.

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Prevention of mortality and pneumonia among institutionalised older adults by dual pneumococcal and seasonal influenza vaccination during a pandemic caused by novel pandemic influenza A (H1N1)

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Introduction: Efficacy of dual vaccination of influenza and pneumococcus in institutionalised older adults remains controversial. In 2009, there was a pandemic by novel pandemic influenza A (H1N1). There was no study performed for dual vaccination in them during an influenza pandemic.

Methods: From December 2009 to November 2010, a prospective 12-month cohort on institutionalised older adults of nine nursing homes was conducted. Elderly persons who had been vaccinated by the Department of Health for seasonal influenza vaccine with or without pneumococcal vaccine but without vaccination for pandemic influenza A (H1N1) were included. Outcome measures included mortality due to all causes, pneumonia, any infective causes and vascular causes.

Results: A total of 457 older residents were included in the study; 246 (53.8%) of them received both seasonal influenza and 23-valent pneumococcal polysaccharide vaccines (PPV-TIV group), and 211 (46.2%) had vaccination of seasonal influenza only (TIV alone group). None of them had vaccination of pneumococcus only. Baseline characteristics including comorbidities and functional status were similar between the groups. The 12-month mortality rates of the PPV-TIV and TIV alone group were 17.1% and 27.0%, respectively. Multivariate analysis demonstrated that dual vaccination in institutionalised older adults significantly reduced all-cause mortality by 46% (hazard ratio=0.54; 95% confidence interval, 0.35-0.84; P<0.01), compared with vaccination of seasonal vaccination alone. Similar and significant efficacy was found in mortality due to pneumonia and any infective causes.

Conclusion: During an influenza pandemic or when the circulating influenza strain was not matched by the trivalent seasonal influenza vaccine, dual vaccination of influenza and pneumococcus provided additional protection to institutionalised older adults in reducing mortality when compared to seasonal influenza vaccination alone.

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Development and validation of a prognostic index for 2-year mortality in Chinese older residents living in nursing home

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Introduction: There is no mortality prediction index for Chinese nursing home older residents. The objective of this study was to derive and validate a 2-year mortality prognostic index for them. It can provide objective prognostication for doctors when counselling patients and their families about treatment options.

Methods: We performed a prospective cohort study on 1120 older residents from 12 nursing homes of Hong Kong. We obtained potential predictors of mortality and performed updated functional assessment. Each risk factor associated independently with 2-year mortality in derivation cohort was assigned a score based on the odds ratio, and risk scores were calculated for each subject by adding the points of risk factor present. Similar analysis was performed on the validation cohort.

Results: Independent predictors of mortality included: aged 86-90 years (3 points); aged ≥91 years (4 points); Charlson comorbidity index ≥4 (6 points); Barthel Index 5-60 (5 points); Barthel Index 0 (10 points); number of hospitalisation in the preceding year (Adbefore) 1 (4 points), Adbefore 2 (5 points), and Adbefore ≥3 (6 points). In the derivation cohort, 2-year mortality was 10.8% in the low-risk group (≤4 points) and 59.9% in the high-risk group (≥14 points). In the validation cohort, 2-year mortality was 11.8% in the low-risk group and 60.4% in the high-risk group. The receiver operating characteristics curve area was 0.761 for the derivation cohort and 0.742 for the validation cohort.

Conclusions: Our prognostic index had satisfactory discrimination and calibration in independent sample of Chinese nursing home older residents. It can be used to identify older residents with high risk for poor outcomes, who need a different level of care.

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Interactive virtual reality Wii in a geriatric day hospital: a study to assess its feasibility, acceptability, and efficacy

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Introduction: Rehabilitation using interactive virtual reality Wii (Wii-IVR) was shown to be feasible in patients with different medical problems, but there was no study looking at its use in geriatric day hospitals (GDHs).

Methods: This was a clinical trial with matched historic-controls. Patients of a GDH were recruited to participate in Wii-IVR by playing 'Wii Fit'. Participants used Wii controller to perform movements involved in arm-ergometer. Each participant received eight sessions of Wii-IVR on top of conventional GDH rehabilitations. Feasibility was assessed by the total time receiving Wii-IVR, percentage maximal heart rate reserve (%MHR) and Borg Perceived Exertion Scale (BS) after participating in Wii-IVR. %MHR and BS were compared with that after performing same duration of arm-ergometer. Acceptability was assessed by an interviewer-administered questionnaire. Efficacy was assessed by comparing improvements in Functional Independence Measure (FIM) between Wii-IVR participants and matched historic-controls, who had received conventional GDH rehabilitations only.

Results: A total of 30 participants with a mean (± standard deviation) age of 80.1 ± 7.1 completed the study. Participants completed a total of 1941 minutes of event-free Wii-IVR. The mean %MHR was 15.9% ± 9.9% and the mean BS was 7.9 ± 2.3. There was no significant difference in %MHR and BS between participating in Wii-IVR and arm ergometer. Most participants found Wii-IVR similar to arm ergometer and would like to continue Wii-IVR if they had Wii at home. Sixty historic-controls that matched in baseline FIM scores, referral diagnoses, age, and gender were chosen for comparison. More improvement in FIM scores was observed in Wii-IVR participants than in historic-controls (7.4 ± 2.5 vs 5.9 ± 3.6; $P<0.05$).

Conclusions: Wii-IVR in GDH is feasible and is welcome by older people. Wii-IVR participants seemed to have more improvement in FIM scores.

Anti-fungal drug usage in haematological patients during a 4-year period in an Asian university teaching hospital

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Introduction: Fungal infection represents a major threat to patients with haematological malignancy, giving rise to significant morbidity and mortality.

Methods: We performed a retrospective analysis on the use of antifungal medications in a 4-year period (2007-2010) in Queen Mary Hospital, Hong Kong. Data were retrieved according to records in the pharmacy, electronic, and paper record.

Results: Bone marrow transplantation was performed in 130 (47%) patients. Antifungal prophylaxis with either oral fluconazole or itraconazole was given in 214 (78%) patients. There were a total of 414 prescriptions of anti-fungal drugs (including liposomal amphotericin B, voriconazole, caspofungin, micafungin, anidulafungin), in which 371 prescriptions were empirical. There were 16 patients with proven invasive fungal infection (IFI), 10 of whom had breakthrough infection while on itraconazole prophylaxis. Interestingly, seven of these cases were due to infection by *Candida* that was itraconazole-sensitive.

Conclusions: One of the major barriers to effective antifungal prophylaxis is probably compliance and absorption of antifungal medications. These results provide important epidemiologic data necessary for the formulation of strategies for prevention and treatment of IFI in Asian patients.

Post-transplant lymphoproliferative diseases in Asian patients: significantly late in onset and favourable response to treatment

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Introduction: Post-transplant lymphoproliferative disease (PTLD) is a serious complication after solid organ transplantation and haematopoietic stem cell transplantation. Its incidence in Asian is not well reported.

Methods: Consecutive cases of PTLD diagnosed within a 10-year period (2001-2010) were analysed. There were no exclusion criteria.

Results: Twenty-one consecutive patients with PTLD in an Asian population were reviewed. The histopathologic diagnoses were monomorphic (CD20-positive diffuse large B-cell lymphoma, n=16); plasmacytic (n=1); Burkitt-like (n=1); natural killer cell lymphoma (n=1); lymphomatoid papulosis (n=1); and classical Hodgkin lymphoma (n=1). Early-onset (<1 year post-transplantation) PTLD constituted only 19% of cases, and all were Epstein-Barr virus (EBV) positive. EBV-negative cases (n=6) developed at a median of 92 (19-170) months, whereas EBV-positive cases occurred much later at 112 (3-230) months. With reduction of immunosuppression followed by local therapy, treatment with the anti-CD20 antibody rituximab with or without combination chemotherapy, complete remission was achieved in 18/21 (86%) of cases. Lactate dehydrogenase level, stage, extranodal involvement, EBV status and International Prognostic Index had no impact on treatment outcome.

Conclusions: EBV-positive PTLD occurred much later in Asian patients. Treatment results of PTLD were favourable.

Shrink-film configurable multi-scale wrinkles for functional cell alignment studies

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We introduce an ultra-rapid, tunable, robust, facile, and inexpensive fabrication method to create multi-scaled biomimetic alignment topography with features ranging from nano- to micro-meters. Commodity plastic shrink-wrap film is oxidised and subsequently shrunk 90% uniaxially for controlled and predictable aligned grooves or 'wrinkles' within minutes. Time-lapse response of human embryonic stem cells (hESCs) to these grooved topographical features is monitored by cytoskeletal and nuclear alignment as well as altered nuclei shape. The alignment of various cell types including pluripotent hESCs as well as the demonstrated functionality of aligned hESC-CM are some examples of how this substrate can be used to rapidly and easily perform otherwise challenging biological studies. The ability to controllably affect contact guidance of hESCs could elucidate critical molecular pathways and lead to directed differentiation into specific lineages. The aligned hESC-CMs provide a more accurate in-vitro model for basic research studies on human cardiac development as well as potential treatment for cardiac injuries. Importantly, such a robust, easy to fabricate and configurable platform (compatible with micro-titer plates and spatiotemporal imaging/mapping) could enable ubiquitous alignment of any adherent cell type for various tissue engineering and injury repair applications.

PIN1 inhibits apoptosis in cancer cells through modulation of anti-apoptotic function of survivin

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Background: PIN1, a peptidyl-prolyl-isomerase, binds a specific motif comprising a phosphorylated serine or threonine preceding a proline (pSer/Thr-Pro) residue in proteins. Through cis-trans isomerisation, it induces conformational changes and modulates functions of many proteins that are involved in cell cycle progression, cell proliferation, and oncogenesis. Previous work has revealed that PIN1 is over-expressed in many cancers including hepatocellular carcinomas (HCC) and contributes to tumour formation. In the current study, the role of PIN1 and the significance of its interaction with survivin, an inhibitor of apoptosis protein (IAP), in evading apopotosis were investigated.

Results and conclusions: Targeted inhibition of PIN1 by small interfering RNA in human cervical carcinoma HeLa and hepatoma PLC/PRF/5 cells enhanced the apoptotic response induced by staurosporine through caspase-3 and caspase-9 activation. Likewise, PIN1 over-expression in HeLa and human non-tumourigenic liver MIHA cells suppressed apoptotic response through inhibition of caspase-3 and caspase-9 activities. In addition, down-regulation of survivin by small interfering RNA in PIN1 over-expressing cells attenuated the anti-apoptotic effect induced by PIN1, suggesting that the inhibition of apoptosis was mediated through the PIN1-survivin interaction. By co-immunoprecipitation assays, PIN1 was found to interact with survivin via the phosphorylated Thr^{34}-Pro motif. Furthermore, PIN1 over-expression enhanced the binding between Thr^{34} phosphorylated survivin, hepatitis B X-interacting protein (HBXIP) and pro-caspase-9. Taken together, these results suggested that the inhibition of apoptosis by PIN1 in cancer cells was partly mediated through the modulation of the anti-apoptotic function of survivin by increasing its binding to pro-caspase-9 via HBXIP in cancer cells.

The adaptor protein APPL1 promotes insulin secretion by modulating expression and complex formation of SNARE proteins

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Objective: Type 2 diabetes is characterised by peripheral insulin resistance and defective insulin secretion from pancreatic islets. Our previous study demonstrated that the adaptor protein APPL1 positively regulates insulin actions in liver and endothelium. In this study, we aimed to investigate whether or not APPL1 regulates pancreatic beta-cell functions using a knockout (KO) mouse model.

Methods: APPL1 KO mice and its wild-type littermates were subjected to high-fat diet feeding for 24 weeks. The glucose metabolism and beta-cell functions were examined in these mice.

Results: APPL1 was abundantly expressed in mouse pancreatic beta cells, but its expression was significantly decreased in obese and diabetic conditions. Genetic ablation of APPL1 resulted in glucose intolerance due to decreased glucose-stimulated insulin secretion in mice. Ex-vivo study showed that pancreatic islets lack of APPL1 exhibited blunted glucose- and potassium-stimulated insulin secretion accompanied by a marked reduction in expression of the exocytotic machinery SNARE proteins and docked insulin granules, while glucose metabolism and calcium mobilisation remained unchanged. On the other hand, these defects were rescued by adenovirus-mediated expression of APPL1 or a constitutively active form of Akt. Furthermore, co-immunoprecipitation assay revealed that APPL1 interacted with syntaxin-1, a key molecule in SNARE protein complex, upon glucose stimulation. Knockdown of APPL1 expression reduced the complex formation of SNARE protein in beta cells, whereas overexpression of APPL1 enhanced this complex formation. Taken together, these results suggest that APPL1 regulates protein expression as well as complex formation of SNARE proteins, thereby controlling insulin secretion in beta cells.

Conclusion: Our study establishes a new role for APPL1 in beta-cell functions, and suggests that the downregulation of APPL1 may contribute to impaired insulin secretion in type 2 diabetes.

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Plasma lipocalin-2 concentration is related to blood pressure and is increased in hypertension

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Introduction: Lipocalin-2 is secreted by adipocytes and is upregulated in obesity. As obesity is known to be a cause of hypertension, we investigated whether the plasma level of lipocalin-2 is related to blood pressure and hypertension.

Methods: The plasma concentration of lipocalin-2 was measured by immunoassay in 1925 subjects of the Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS). Blood pressure was measured after prolonged resting by a trained nurse manually using a calibrated sphygmomanometer three times at 5-minute intervals.

Results: Plasma lipocalin-2 level was higher in men than in women (median [IQR] 37.7 [30.5-47.9] vs 31.6 [25.4-40.4], P<0.001). It was significantly related to age (r=0.15, P<0.001) and systolic blood pressure (r=0.15, P<0.001). In women but not in men, it was also significantly related to waist circumference (r=0.16, P<0.001), body mass index (r=0.09, P=0.004), diastolic blood pressure (r=0.14, P<0.001) and fasting plasma glucose (r=0.089, P=0.004). Plasma lipocalin-2 level was significantly higher (P<0.001 adjusted for age) in hypertensive men and women (median [IQR], 41.1 [31.7-53.0] and 36.5 [27.5-50.1]) compared to normotensive men and women (36.9 [29.6-45.6] and 30.9 [25.2-38.3]).

Conclusion: Plasma lipocalin-2 concentration is related to systolic blood pressure, and is higher in men and in people with hypertension. Lipocalin-2 may be involved in the pathogenesis of hypertension.

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Implication of the type 2 diabetes susceptibility loci identified in genome-wide association studies: long-term follow-up studies in Southern Chinese

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Objective: Genome-wide association (GWA) studies have led to the identification of novel susceptibility loci which showed promising associations with type 2 diabetes (T2DM). This project aimed to establish the role of these novel T2DM-susceptibility loci in Southern Chinese.

Methods: Seventeen T2DM-associated single nucleotide polymorphisms (SNPs) were examined for their associations with glycaemic progression in an 8-year follow-up study based on subjects from the CRISPS cohort. Three SNPs which showed potential associations (P<0.1) with glycaemic progression were further evaluated for associations with T2DM development in a 12-year follow-up study.

Results: In the 8-year follow-up study for glycaemic progression involving 518 glycaemic progression cases and 998 persistent normal glucose tolerance (NGT) controls, the combined genetic risk score of these SNPs showed an odds ratio (OR) of 1.07 ($P=1.3x10^{-3}$) for each additional risk allele. Moreover, the *CDKN2A/B* rs10811661 was significantly associated with glycaemic progression (OR=1.19; P=0.026). Trends for associations with glycaemic progression were also observed in *KCNJ11* rs5219 (OR=1.17; P=0.051) and *IGF2BP2* rs11711477 (OR=1.17; P=0.086). In the 12-year follow-up study for T2DM development involving 200 incident T2DM cases and 903 persistent NGT controls, *CDKN2A/B* rs10811661 showed a significant association with incident T2DM (OR=1.42; $P=2.3x10^{-3}$) which persisted after adjustment for confounding factors.

Conclusions: This study has demonstrated the combined genetic effect of the T2DM-associated SNPs, identified from GWA studies, on glycaemic progression in Southern Chinese. *CDKN2A/B* rs10811661 which showed an independent association with T2DM development warrants further investigation.

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Efficacy and safety of single agent sunitinib in treating advanced hepatocellular carcinoma patients after sorafenib failure: a prospective, open-label, phase II study

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Background: This is an open-label and single-arm phase II study to assess the efficacy and tolerability of sunitinib for the treatment of sorafenib-refractory advanced hepatocellular carcinoma (HCC) patients.

Methods: Between October 2008 and October 2010, eligible patients with advanced HCC and documented disease progression after sorafenib treatment received sunitinib 37.5 mg continuously at Queen Mary Hospital, Hong Kong. Response assessment was performed after every 8 weeks. The primary endpoint was time-to-progression (TTP) and the secondary endpoints were tumour response rate (RR), overall survival (OS), and safety.

Results: At the time of analysis, 38 patients were recruited in the trial. The median age was 56 (range, 27-80) years and all patients were in ECOG Performance Status 0-1. A total of 95% of patients were chronic hepatitis B carriers with underlying Child-Pugh A and B cirrhosis in 70% and 30% of the enrolled patients, respectively. Ten (25%) patients had tumour vascular invasion and 32 (80%) patients had extra-hepatic metastasis. Among 35 evaluable patients, RR was 6% with two patients achieved partial response and another 12 (34%) patients achieved stable disease. Overall, 40% of patients derived clinical benefits from sunitinib treatment for at least 8 weeks. The median TTP was 2.9 (0.5-15) months and OS was 5.2 (1-22.5) months. Malaise (60%), neutropenia (45%), and diarrhoea (36%) were the most commonly encountered adverse events, with nearly 30% of patients experienced grade 3 or 4 toxicity. No treatment-related death was reported.

Conclusions: Sunitinib has substantial anti-tumour activity with manageable toxicity profile in treating sorafenib-refractory advanced HCC population. These data may imply sunitinib inhibits signalling pathways involved in sorafenib resistance and support the hypothesis of sequential use of antiangiogenic tyrosine kinase inhibitors in treating advanced HCC patients.

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The use of single agent sorafenib in the treatment of advanced hepatocellular carcinoma patients with underlying Child-Pugh B liver cirrhosis

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Background: Hepatocellular carcinoma (HCC) is a common malignancy especially in patients with chronic liver disease. It often presents late. Sorafenib is the only systemic treatment for advanced HCC proven to have survival benefit. Previous studies included predominantly patients with Child-Pugh A liver cirrhosis, and the use of sorafenib in patients with poor liver function is controversial. This study aimed to explore the efficacy and tolerability of using sorafenib in Child-Pugh B patients.

Methods: Advanced HCC patients treated with sorafenib at Queen Mary Hospital, Hong Kong were analysed retrospectively. Treatment outcomes were analysed according to their respective Child-Pugh status.

Results: The baseline demographic parameters were comparable between 108 Child-Pugh A and 64 Child-Pugh B patients. Both clinical benefit rate (21.3% vs 25.0%; P=0.58) and progression free survival (median, 3.2 vs 2.8 months; P=0.31) were similar between the two groups. The overall survival was significantly longer in Child-Pugh A patients (median, 6.1 vs 3.9 months; P=0.009). The most common grade 3/4 adverse events (AEs) were hand-foot-syndrome (13.5%), diarrhoea (9.9%), and rash (7.0%). Grade 3/4 leukopenia, thrombocytopenia, and anaemia occurred in 2.9%, 5.3%, and 8.8% of the patients, respectively. Child-Pugh A and B patients experienced similar incidence of most AEs. Nonetheless, Child-Pugh B patients experienced more anaemia (71.4% vs 50.5 %; P=0.01), gastrointestinal bleeding (15.6% vs 5.6%, P=0.05) and hepatic encephalopathy (10.9% vs 1.9%; P=0.01).

Conclusions: Child-Pugh A and B patients tolerated sorafenib similarly and derived comparable clinical and progression-free survival benefit. Child-Pugh B patients were more susceptible to developing cirrhotic complications, thus vigilant surveillance is needed.

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Non-traditional biomarkers in the prediction of cardiovascular events among Chinese

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Introduction: Biomarkers of subclinical systemic chronic inflammation are increasingly recognised as a key player in atherosclerosis. C-reactive protein, measured using high-sensitivity assay (hsCRP), is the most promising inflammatory marker in predicting the risk of cardiovascular diseases (CVD). As obesity is associated with disregulated expression of various adipokines, either pro-inflammatory or anti-inflammatory, such adipokines may also serve as non-traditional biomarkers for the accelerated atherosclerosis associated with obesity. In this prospective cohort study, we examined the predictive value of a variety of non-traditional biomarkers for CVD among Hong Kong Chinese, and determined if they would enhance the predictive value in conjunction with the traditional markers.

Methods: Subjects were recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study 2 (CRISPS 2) cohort. Those with known cardiovascular disease(s) were excluded. Baseline serum levels of hsCRP, IL-6, soluble tumour necrosis factor alpha receptor 2 (sTNF-αR2; a surrogate marker of TNF-α), and adiponectin were determined. Subjects were followed prospectively for 6 years.

Results: A total of 1776 subjects were included in the final analysis. The cumulative incidence of CVD was 85 (4.8%). At baseline, subjects with incident CVD had higher proportions of male gender and current/former smoker. They were older, and had higher body mass index (BMI), waist circumference (WC), blood pressure (BP), HOMA-IR, and fasting glucose levels (all P<0.001), compared to those who did not develop CVD (non-CVD). They also had higher LDL-cholesterol and triglycerides, and lower HDL-cholesterol levels. Among the non-traditional biomarkers, subjects with incident CVD had higher baseline levels of hsCRP (1.50 vs 0.69 mg/L), IL-6 (0.83 vs 0.56 pg/mL) and sTNFR2 (2276 vs 1879 ng/mL) [all P<0.001], but similar adiponectin levels, compared to non-CVD subjects. Cox proportional hazards regression showed that baseline hsCRP, IL-6, and sTNFR2 were independent predictors of incident CVD even after controlling for the established risk factors.

Conclusion: In this 6-year prospective study, hsCRP, IL-6, and sTNFR2 were independent predictors of incident CVD in Hong Kong Chinese, in addition to the established CVD risk factors. Measurements of these non-traditional biomarkers may allow early CVD risk stratification among these low-risk, apparently healthy subjects.

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Improvement in diagnostic delay in spondyloarthritis patients over time but disease control is still inadequate

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Introduction: Diagnostic delay has been investigated in a previous study. Potential risk factors including: incomplete clinical picture, non-radiographic sacroiliitis, and female sex were found to be associated with diagnostic delay. Our study was to investigate factors associated with diagnostic delay in the local group of spondyloarthritis (SpA) patients.

Methods: A total of 127 SpA patients of Chinese ethnicity were recruited from the rheumatology clinic of Queen Mary Hospital from 2007 to 2008. All of them were diagnosed to have SpA according to expert opinion. Recruited patients were interviewed for the delay in SpA diagnosis. Clinical and radiological features were compared among the significant delayed group (delay in diagnosis of more than 5 years) and the less delayed group (delay of less than 5 years). Variables found to be different were used as independent variables in the multivariate regression using delay in diagnosis as the dependent variable.

Results: The delay in SpA diagnosis was found to be associated with age (Beta=0.17; P=0.04) and disease duration (Beta=0.51; P<0.01). The significantly delayed group and the less delayed group were not different in terms of disease activity (4.0±1.9 in the delayed group and 4.1±1.8 in the less delayed group; P=0.72). The recruited patients had high disease activity (mean Bath Ankylosing Spondylitis Disease Activity Index, 4.0).

Conclusion: Less diagnostic delay was observed in the recent years. Despite the finding, most of the SpA remains inadequately treated.

Mass spectrometric method for the rapid characterisation of embryonic stem cell differentiation

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Introduction: Differentiation of human embryonic stem cells (hESCs) is an invaluable model for the investigation of molecular events during embryonic development, and supplies functional differentiated cells for regenerative therapies. Identification of 'molecular signatures' specific for different stages of lineage-specific differentiation of hESCs will provide a quantitative method for quality assurance of this process.

Methods: A novel approach based on Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF-MS) for the characterisation of the differentiation of hESC into cardiomyocytes (CM) was proposed. Buffer washed cells were directly mixed with a matrix solution and subsequently deposited onto the stainless steel target for MALDI analysis. The resulting mass spectrometric (MS) profiles were highly reproducible and can be used to reflect cell phenotypes such as apoptosis1 and cell cycle stages. As few as 1500 cells can be used in this analysis.

Results: Selected MS features generated from different cell types were highly distinctive, and can be used as signatures for hESC and differentiating CM.

Conclusion: Utilisation of intact cell MALDI mass spectrometry in studying hESC differentiation can be used as a basis for the development of a reliable, fast, label-free and high throughput method for barcoding hESC differentiation.

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Distinct roles of MicroRNA-1 and -499 in ventricular specification and functional maturation of human embryonic stem cell–derived cardiomyocytes

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Background: 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.

Methods and Results: We hypothesised that miRs that figure prominently in cardiac differentiation are differentially expressed in differentiating, developing, and terminally mature human cardiomyocytes (CMs). As a first step, we mapped the miR profiles of human (h) embryonic stem cells (ESCs), hESC-derived (hE), fetal (hF) and adult (hA) ventricular (V) CMs. 63 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 non-cardiac specific. 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 APD and hyperpolarized RMP/MDP in hE-VCMs due to increased Ito, IKs and IKr, and decreased If ($P<0.05$) as signs of functional maturation. Also, LV-miR-1 but not -499 augmented the immature Ca^{2+} transient amplitude and kinetics. Molecular pathway analyses were performed for further insights.

Conclusion: We conclude that miR-1 and -499 play differential roles in cardiac differentiation of hESCs in a context-dependent fashion. While miR-499 promotes ventricular specification of hESCs, miR-1 serves to facilitate electrophysiological maturation.

Fibroblast growth factor 21 acts as a negative feedback regulator to suppress the thermogenic activities of brown adipose tissue in mice

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Introduction: Fibroblast growth factor 21 (FGF21), a pleiotropic hormone-like protein, has been suggested as an important regulator of energy metabolism and systemic insulin sensitivity in animals. In both humans and animals, circulating levels of FGF21 are elevated in obesity. However, the role and underlying mechanism of FGF21 on energy expenditure and adiposity remains poorly characterised. This study aimed to address this question using FGF21 knockout (KO) mice.

Methods: FGF21 KO mice and their wild-type (WT) littermates were subjected to standard chow or high fat diet for 24 weeks. Basic metabolic parameters were monitored accordingly. The mitochondria biogenesis, thermogenesis and lipid profile were examined in FGF21 KO mice and WT littermates under cold exposure or not. The underlying molecular basis was explored by explant study.

Results: Compared to WT littermates, FGF21 KO mice exhibited a higher metabolic rate and core body temperature and were resistant to diet- and ageing-induced obesity. Histological examination of interscapular brown adipose tissue (BAT) and white adipose tissue (WAT) in FGF21 KO mice revealed smaller adipocytes with increased mitochondrial density. In response to cold challenge, FGF21 expression was markedly elevated in BAT, but not liver. Furthermore, cold-induced decline of core body temperature in FGF21 KO mice was much slower. At the molecular level, the expression of key genes involved in mitochondria biogenesis, thermogenesis, lipolysis and lipid β-oxidation was elevated in FGF21 KO mice compared to WT littermates. Our ex-vivo studies in isolated brown adipose tissue demonstrated that recombinant FGF21 inhibited CL316,243-induced oxygen consumption through protein kinase B, which was accompanied by the suppressed production of cyclic adenosine monophosphate (cAMP).

Conclusion: These findings suggest that FGF21 produced from BAT may act as an autocrine factor to suppress the thermogenesis, which in turn decreases energy expenditure and accelerates obesity.

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Cellular mechanisms involved in intermittent hypoxia-induced heart damage in rat

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Background: Obstructive sleep apnoea (OSA), characterised by intermittent hypoxia (IH) during sleep, is increasingly recognised as an independent risk factor of cardiovascular diseases (CVD). OSA has been reported to be associated with changes in the levels of circulating oxidative stress and inflammatory markers as well as dyslipidemia, supporting their mediating roles in cardiovascular pathogenesis. This study aimed to investigate the effect of IH on heart tissue using an IH-exposed rat model and to explore the potential mechanisms involved in the occurrence of cellular injury in the heart.

Methods and Results: Male Sprague–Dawley rats were divided into intermittent air (IA)- or IH-exposed groups, and sacrificed after 4 weeks. IH caused elevations in serum malondialdehyde (MDA) and cytokine-induced neutrophil chemoattractant-1 (CINC-1) and reduction in serum adiponectin levels. In contrast, cardiac oxidative stress and pro-inflammatory markers were suppressed while cardiac adiponectin and cholesterol levels were elevated after IH exposure. In parallel, there was an increase in apoptosis in the heart tissue of IH-exposed rats, demonstrated by TUNEL staining and elevations of Bax and cleaved caspase-3 protein. Myocardial damage was further evident with decreased arterial vessel and capillary densities, increased cardiac fibrosis and the loss of troponin I.

Conclusions: Our data demonstrated for the first time that IH exposure caused systemic oxidative and inflammatory responses but "protective" responses in heart tissue. Despite such a local compensatory protective mechanism, heart damage was still observed that may have likely resulted from IH-induced caspase-dependent apoptosis cell death via cholesterol accumulation in the heart.

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UCP4 is a target effector of NF-κB c-Rel pro-survival pathway against oxidative stress

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Objectives: To examine the role of the cRel, subunit of NF-κB, involved in the protective mechanism of UCP4 in alleviating mitochondrial dysfunction and oxidative stress.

Methods: c-Rel construct was co-transfected with UCP4-promoter-luciferase-fusion expression vector into SH-SY5Y cells. Luciferase activity was measured to determine the promoter activity of UCP4. Changes in protein expression of UCP4 were measured by western blot. Binding of c-Rel on UCP4 promoter region was shown by gel-shift assay (EMSA). Superoxide levels were measured by DHE staining. Reduced, oxidised glutathione and mitochondrial membrane potential (MMP) were measured after H_2O_2 treatment.

Results: We showed that c-Rel overexpression induced NF-κB activity without affecting p65 levels. Overexpression of c-Rel increased UCP4 promoter activity and protein expression. Electrophoretic mobility shift assay showed increased specific binding of c-Rel protein complexes to a NF-κB site on the UCP4 gene promoter. Under H_2O_2-induced oxidative stress, UCP4 knockdown significantly increased superoxide levels, decreased GSH (reduced glutathione) and increased GSSG (oxidised glutathione) levels, compared to controls. UCP4 expression induced by c-Rel overexpression significantly decreased superoxide levels, and preserved GSH levels and MMP under similar stress.

Conclusions: In conclusion, our findings demonstrate the link between UCP4 and NF-κB c-Rel against oxidative stress in an in-vitro model of oxidative stress. We have shown that UCP4 can exert protective effects against H_2O_2 by being up-regulated by c-Rel overexpression, thereby reducing ROS levels, preserving cellular GSH levels and maintaining MMP. The protective effects of c-Rel overexpression were significantly decreased after UCP4 knockdown. These findings demonstrated that UCP4 is a target effector of the NF-κB c-Rel pro-survival pathway, and that UCP4 may act as a mitochondrial surveillance factor that mitigates the effects of oxidative stress through activation of this pathway.

Endothelial progenitor cells derived from human-induced pluripotent stem cells, embryonic stem cells, and bone marrow stem cells in attenuation of limb ischaemia in mice

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Background: Human-induced pluripotent stem cells (hiPSC) have the capacity to differentiate into multiple lineages and provide an unlimited pool of cells for autologous cell replacement therapies. The presents study aimed to compare the in-vitro and in-vivo angiogenic effects of endothelial-like cells (ECs) derived from human bone marrow mononuclear cells (BM-MNC), human embryonic stem cells (hESCs) and human-induced pluripotent stem cells (hiPSCs).

Methods: We differentiated functional ECs from BM-MNC(BM-ECs), hESCs(hESC-ECs) and hiPSCs(hiPSC-ECs), and compared their in-vitro tube formation, migration function and cytokine expression profiles, and in-vivo capacity to attenuate mice hind-limb ischaemia.

Results: Differentiation of BM-EC from BM-MNC was only achieved in 1/6 (17%) patients with coronary artery disease. Nevertheless, BM-ECs, hESC-ECs and hiPSC-ECs exhibited typical cobblestone morphology, the ability of DiI-Ac-LDL dye uptake, and Ulex europaeus lectin antigen expression. In-vitro functional assay demonstrated that hiPSC-ECs and hESC-ECs had similar capacity for tube formation and migration as BM-ECs (all $P>0.05$). During hypoxia, increased expression of major angiogenic factors including epidermal growth factor, hepatocyte growth factor, vascular endothelial growth factor, placental growth factor and stromal derived factor-1 were observed in all ECs cultures as compared with normoxia (all $P<0.05$). Compared with medium only, transplanting BM-ECs (n=8), hESC-ECs (n=8) or hiPSC-ECs (n=9) into mice significantly attenuated severe hind-limb ischaemia via enhancement of neovascularisation.

Conclusions: Functional ECs can be generated from different cell sources for attenuation of severe hind-limb ischaemia. However, differentiation of BM-ECs from BM-MNC was achieved in only a small proportion of patients, and hESC-ECs or iPSC-ECs are more ready available as 'off-the-shelf' format for the treatment of tissue ischaemia.

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Development of a prototype catecholamine-O-methyltransferase cell-ELISA system for the assay of chemicals of estrogenic potential

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Introduction: Estrogens are not only capable of regulating endocrine and reproductive systems, they also modulate neuronal development in the foetus and neonate and cognitive function in adults. Estrogens do not necessarily closely resemble the female hormone, estradiol (E2), in chemical structure, and therefore are not easily recognised. Chemicals such as bisphenol-A (BPA), alkyl phenols, phthalate esters are found in foods packaged with plastic materials and a wide range of other everyday goods. They have teratogenic and feminising effects at low levels in rats and fish. Many new chemicals are being introduced by industry, the estrogenic potential of which has not been assessed. Current tests of estrogenic potential rely on multigenerational rat assays, which are time-consuming and expensive.

Methods: We developed a sensitive and versatile assay to assess estrogenic potential. The assay was comprised of two parts, the suppression of expression of catecholamine-O-methyltransferase (COMT) in MCF-7 (human breast cancer) cells and the subsequent assay of COMT by an enzyme-linked immunosorbent assay (ELISA). This novel ELISA will use protein lysates extracted from MCF-7 cells that express COMT, to detect effects of estrogen or its mimics. Future application can extend to other potential cell lines as COMT expressers including SH-SY5Y (human neuronal) and HepG2 (human liver) to extend the assay's physiological detection range, because these cell lines use other coactivators of the E2 receptor. Our assays utilised recombinant COMT protein (COMT-FLAG) used as competitor in our standards and the recombinant COMT-NE used as the detection antigen. "NE" is a novel short protein tag we designed to serve as a detection epitope (patent pending) in our kit.

Results: Treatment of estrogen or BPA in MCF-7 cells significantly and dose-dependently decreased COMT expression as shown in both western blots and our novel ELISA assay.

Conclusion: There is increasing awareness of exposure to endocrine-disrupting environmental chemicals that are capable of exerting estrogenic effects. Exposure to environmental contaminant BPA shows estrogenic endocrine disruption in this study. Reduction in COMT expression by BPA may explain how BPA contributes to increased tumour formation, presumably via estrogen-mediated carcinogenesis.

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Adipocyte fatty acid binding protein potentiates toxic lipids-induced endoplasmic reticulum stress via its inhibition of autophagy

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Introduction: Chronic inflammation is the key link between obesity and its related cardio-metabolic complications. Endoplasmic reticulum (ER) stress is the potent trigger of inflammation in obese adipose tissue. However, the mechanism that links ER stress with inflammation is unclear. Adipocyte fatty acid binding protein (A-FABP) has been shown to mediate endotoxin-induced inflammation in macrophages by forming a positive loop with c-Jun-N terminal kinase (JNK) which is the downstream regulator of ER stress. This study aimed to examine the role of A-FABP in association with autophagy in potentiating toxic lipids-induced ER stress in macrophages.

Methods: RAW264.7 macrophages with adenovirus-mediated over-expression of A-FABP or luciferase, and primary macrophages derived from A-FABP knockout mice or their WT littermates were treated with palmitic acid (PA) or vehicle. RAW264.7 macrophages were transfected with siRNA of autophagic protein Atg7 or scramble RNA followed by the stimulation of PA. The autophagic flux, mRNA and protein expression of ER stress markers, autophagic proteins and inflammatory markers were determined by real-time quantitative PCR and Western blot analysis.

Results: Adenovirus-mediated over-expression of A-FABP reduced the expression of autophagic proteins Atg7 and beclin-1, and this change was accompanied by enhanced ER stress and inflammation in response to PA stimulation. Both basal and PA-induced autophagic flux and protein expression were enhanced while ER stress was reduced in macrophages derived from A-FABP knockout mice comparing to wild type macrophages. Knocking down of Atg7 leading to defective autophagy elevated PA-induced ER stress in macrophages. Treatment of macrophages with JAK2 inhibitor AG490 also reduced the autophagic protein expression, but further enhanced the ER stress in response to PA stimulation.

Conclusion: A-FABP potentiates toxic lipids-induced ER stress through inhibition of autophagy which may possibly via its attenuation of JAK2 signalling pathway in macrophages.

Effects of functional transient receptor potential channels on adipogenesis and proliferation in human preadipocytes

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Background: Preadipocytes are extensively used as a type of proliferative cell culture model to investigate proliferation and differentiation of adipocytes and lipodystrophy (eg obesity)-related metabolic dysfunctions and disorders. However, cell biology is not well understood in human preadipocytes. The present study was to investigate the expression of transient receptor potential (TRP) channels in human preadipocytes, and their role in regulating adipogenesis and proliferation.

Methods: Whole-cell patch voltage-clamp, RT-PCR, Western blot, and confocal microscopic approaches were used to determine functional expression of TRP channels in cultured human preadipocytes. ShRNA targeting TRP channels were constructed to silence the related TRP channels. Adipogenesis and oil red O staining were applied to observe the effect of the TRP channels on cell differentiation. Cell proliferation assay was made with MTT and ^{3}H-thymidine incorporation approaches.

Results: A small background current was inhibited by the TRPC channel blocker La^{3+}. Removal of Mg^{2+} of pipette solution or bath solution induced a Mg^{2+}-sensitive current, and the current was suppressed by the TRP channel blocker 2-aminoethoxydiphenyl borate. In addition, an intracellular Ca^{2+}-activated current was inhibited by the TRPV channel blocker capsazepine. RT-PCR revealed significant mRNA expression of TRPC1, TRPC4, TRPV2, TRPV4, and TRPM7 channels in human preadipocytes. Western blot analysis confirmed the protein expression of these TRP channels. Interestingly, shRNAs targeting TRPV2, TRPV4 and TRPM7 suppressed the corresponding gene and protein expression, and significantly reduced adipogenesis of human preadipocytes, which was revealed by the reduced oil red O staining and the decreased expression of peroxisome proliferator-activated receptor gamma (PPARγ, a marker of adipogenesis). Proliferation of human preadipocytes was reduced by TRPV2-shRNA, TRPV4-shRNA and TRPM7-shRNA.

Conclusion: Our results demonstrate for the first time that multiple TRP channels, TPC1/4, TRPV1/2/4, and TRPM7, are present in human preadipocytes. TRPV2, TRPV4 and TRPM7 channels participate in regulating adipogenesis and proliferation.

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Adipocyte-selective disruption of SIRT1 accelerates high fat diet– and ageing-induced insulin resistance by inducing cellular senescence in mice

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Objective: SIRT1 is an NAD^+-dependent histone deacetylase that antagonises ageing-associated diseases in multiple tissues. However, the physiological function of SIRT1 in adipocytes remains unknown. This study sought to address whether and how adipocyte SIRT1 modulates the systemic energy homeostasis and insulin resistance.

Methods: Adipocyte-selective knockout mice of SIRT1 (AKO) were obtained by crossing the $Sirt1^{flox/flox}$ mice with the adipocyte-specific Cre transgenic mice (aP2-Cre). $Sirt1^{flox/flox}$ mice were used as the wild type (WT) control. Basic metabolic parameters including body weight, food consumption and the levels of blood glucose and lipids were monitored bi-weekly. Glucose tolerance and insulin sensitivity were evaluated by glucose tolerance test and insulin tolerance test. Serum concentration of insulin and adiponectin was quantified by in-house immunoassays. The extent of pro-inflammatory cytokine expression and immune cell infiltration were determined by quantitative PCR (qPCR). The adipose tissue senescence was assessed by β-galactosidase staining and qPCR analysis for senescence-related genes $p16^{INK4A}$ and $p21^{WAFI}$. The relative telomere length in primary adipocytes was examined by flowcytometry based fluorescence in-situ hybridisation (flow-FISH).

Results: WT mice and AKO mice had similar levels of body weight and food intake. However, adipocyte-specific deletion of SIRT1 accelerated high fat diet– and ageing- induced glucose intolerance and insulin resistance in mice. The exacerbation of high fat diet evoked insulin resistance in AKO mice was further corroborated by the impaired insulin signalling in peripheral tissues including liver and muscle. Moreover, a higher level of ectopic fat accumulation was observed in AKO mice compared to the WT control. A more active inflammation status was observed in SIRT1 deficient adipose tissues, exemplified by a higher extent of immune cell infiltration and elevated expression of proinflammatory cytokines. Mechanistically, ablation of SIRT1 in adipocytes caused a more pronounced senescence-like phenotype of adipose tissues in mice upon high fat diet or ageing. Flow-FISH analysis demonstrated that SIRT1 deficient adipocytes had a shorter telomere compared to that in the intact adipocytes.

Conclusion: SIRT1 maintains the stability of telomere in adipocytes, which in turn protects the adipose tissue from high fat diet– and ageing-induced senescence and malfunctioning. Senescent adipose tissue, characterised by elevated inflammation and decreased adiponectin secretion, eventually leads to the derangement of systemic energy homeostasis.

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Prevention of acute myocardial infarction and stroke among elderly persons by dual pneumococcal and influenza vaccination: a prospective cohort study

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Background: Despite World Health Organization recommendations, the rate of 23-valent pneumococcal (PPV) and influenza (TIV) vaccination among elderly persons in Hong Kong, China, is exceptionally low because of doubts about effectiveness of vaccination. The efficacy of dual vaccination remains unknown.

Methods: From 3 December 2007 to 30 June 2008, we conducted a prospective cohort study by recruiting outpatients aged ≥65 years with chronic illness to participate in a PPV and TIV vaccination programme. All were observed until 31 March 2009. The outcome of subjects, including the rates of death, hospitalisation, pneumonia, ischaemic stroke, acute myocardial infarction, and coronary and intensive care admissions, were determined.

Results: Of the 36 636 subjects recruited, 7292 received both PPV and TIV, 2076 received TIV vaccine alone, 1875 received PPV alone, and 25 393 were unvaccinated, with a duration of follow-up of 45 834 person-years. Baseline characteristics were well-matched between the groups, except that there were fewer male patients in the PPV and TIV group and fewer cases of comorbid chronic obstructive pulmonary disease among unvaccinated persons. At week 64 from commencement of the study, dual-vaccinees experienced fewer deaths (hazard ratio [HR], 0.65; 95% confidence interval [CI], 0.55-0.77]; P<0.001) and fewer cases of pneumonia (HR, 0.57; 95% CI, 0.51-0.64; P<0.001), ischaemic stroke (HR, 0.67; 95% CI, 0.54-0.83; P<0.001), and acute myocardial infarction (HR, 0.52; 95% CI, 0.38-0.71; P<0.001), compared with unvaccinated subjects. Dual vaccination resulted in fewer coronary (HR, 0.59; 95% CI, 0.44-0.79; P<0.001) and intensive care admissions (HR, 0.45; 95% CI, 0.22-0.94; P=0.03), compared with among unvaccinated subjects.

Conclusions: Dual vaccination with PPV and TIV is effective in protecting elderly persons with chronic illness from developing complications from respiratory, cardiovascular, and cerebrovascular diseases, thereby reducing hospitalisation, coronary or intensive care admissions, and death.

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33 Convalescent plasma treatment reduced mortality in patients with severe pandemic influenza A (H1N1) 2009 virus infection

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Background: Experience from treating patients with Spanish influenza and influenza A(H5N1) suggested that convalescent plasma therapy might be beneficial. However, its efficacy in patients with severe pandemic influenza A(H1N1) 2009 virus (H1N1 2009) infection remained unknown.

Methods: During the period from 1 September 2009 through 30 June 2010, we conducted a prospective cohort study by recruiting patients aged ≥18 years with severe H1N1 2009 infection requiring intensive care. Patients were offered treatment with convalescent plasma with a neutralising antibody titer of ≥1:160, harvested by apheresis from patients recovering from H1N1 2009 infection. Clinical outcome was compared with that of patients who declined plasma treatment as the untreated controls.

Results: Ninety-three patients with severe H1N1 2009 infection requiring intensive care were recruited. Twenty (21.5%) patients received plasma treatment. The treatment and control groups were matched by age, sex, and disease severity scores. Mortality in the treatment group was significantly lower than in the non-treatment group (20.0% vs 54.8%; P=0.01). Multivariate analysis showed that plasma treatment reduced mortality (odds ratio [OR], 0.20; 95% confidence interval [CI], 0.06-0.69; P=0.011), whereas complication of acute renal failure was independently associated with death (OR, 3.79; 95% CI, 1.15-12.4; P=0.028). Subgroup analysis of 44 patients with serial respiratory tract viral load and cytokine level demonstrated that plasma treatment was associated with significantly lower day 3, 5, and 7 viral load, compared with the control group (P<0.05). The corresponding temporal levels of interleukin 6, interleukin 10, and tumour necrosis factor α (P<0.05) were also lower in the treatment group.

Conclusions: Treatment of severe H1N1 2009 infection with convalescent plasma reduced respiratory tract viral load, serum cytokine response, and mortality.

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34 A retrospective review of horse versus rabbit anti-thymocyte globulin in treatment of severe aplastic anemia in a single institution

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Introduction: Anti-thymocyte globulin (ATG) and cyclosporine has been a standard first-line treatment of severe aplastic anaemia (SAA). A recent publication reported a significant worse outcome in patients treated with rabbit ATG compared with horse ATG. We therefore conducted this retrospective review of SAA patients in our unit between January 2006 and June 2011 and compared the treatment outcome of patients treated with these two ATG.

Methods: Consecutive adult patients diagnosed with SAA in our unit are recruited into this review. There are eight patients treated with ATG in our unit during study period. Rabbit and horse ATG as first-line treatment were each given in four patients. Data on demographics and haematological response were collected. Haematological response is defined as no longer fulfilling the diagnostic criteria of SAA.

Results: The bone marrow of all these eight patients were markedly hypocellular at diagnosis, meeting the diagnostic criteria of SAA. After a median follow-up of 661 days (124-1876 days), 50% of patients in rabbit ATG group had haematologcial response. After a median follow-up of 270 days (110-954 days), 25% of patients in horse ATG group had haematological response. One patient in horse ATG arm died of sepsis 4 months after treatment and the remaining two patients required second-line treatment with rabbit ATG. Two patients in rabbit ATG group while one patient in horse ATG group developed diabetes mellitus.

Conclusion: Rabbit ATG compared favourably with horse ATG as first-line treatment of SAA in our patients.

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Laser- and chemical-induced fusion to reverse engineer the physical size of human embryonic stem cells–derived cadiomyocytes: a nongenetic approach for driven physiological hypertrophy and maturation

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Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs) can self-renew while maintaining their pluripotency to differentiate into cadiomyocytes (CMs), providing a potential unlimited source of donor cells for replacement therapies. However, substantial hurdles remain. For instance, hESC-CMs have small physical size (~10x<adult CMs) and immature functional properties. Adult CMs are bi- or multi-nucleated, ~200 micrometers in length and ~2-300pF in size; by contrast, hESC-CMs are typically ~10-15 times smaller, often mono-nucleated, and do not grow in physical size by undergoing physiological hypertrophy even after long-term culturing (>150 days). Indeed, bi-nucleation of CMs has been suggested as an evolutionary adaptive response in metabolically active cells to double RNA for protein synthesis. Developmentally, bi-nucleated CMs arise from the absence of cytokinesis after karyokinesis during the final round of (incomplete) cell division, followed by physiological hypertrophy. This contrasts the fusion of skeletal muscle myoblasts to form multi-nucleated myotubes. Here, we pursued a combination of laser- and chemical-based induced fusion to reverse-engineer physiological hypertrophy of hESC-CMs for increasing their physical size. By employing optical tweezers that exploit a focused laser microbeam platform, we fused hESC-CMs to construct larger bi-nucleated hESC-CMs in a multi-step process: First, two optical traps were utilised to position two hESC-CMs such that their point of contact is located at the laser scissors' cutting spot. Upon irradiation, an open fusion pore forms then enlarges, with the membranes merging and the fused product gradually rounding up to become a "heterokaryon". Similar outcomes could be accomplished by polyethylene glycolinduced fusion of LV-MLC2v-GFP- and tdTomato-labeled hESC-CMs to generate hypertrophied yellow heterokaryons. Electrophysiological experiments further showed signs of Ca-handling maturation. Not only do our results shed developmental insights but generation of adult-like cells without genetic manipulations is of pragmatic significance for translating into cell-based therapies and other applications (eg, accurate disease modeling, cardiotoxicity and drug screening).

Impact on absolute mortality due to intensive glucose lowering for patients with diabetes

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Introduction: Intensive control of blood glucose in diabetic patients is associated with suboptimal survival.[1] In the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial, 10 104 patients were randomised to intensive or standard therapy (target glycated haemoglobin levels <6.0% vs 7-7.9%) for a mean treatment duration of 3.7 years.[2] To properly appreciate the implications of this trial's findings, they should be viewed in absolute terms.

Methods: Relevant results reported in the ACCORD trial were therefore used to calculate unadjusted number needed to treat (NNT) and relative risk (RR) values and their 95% confidence intervals (CIs), as described previously.[3]

Results: Fatal event rates expressed as unadjusted RR and NNT/year values together with their respective 95% CIs, are shown in the Table.

Conclusion: Intensive glucose lowering was associated with a small but statistically significant negative NNT/year that amounts to a "number needed to harm". This contrasts with the mortality benefit (NNT/year of +163),[3] which accrued from simvastatin therapy in the high-risk patients reported in 4S (Scandinavian Simvastatin Survival Study). In terms of all-cause mortality, the potential harm from such intensive glucose lowering is a matter of concern.

Deaths	RR (95% CI)	NNT/year (95% CI)
From any cause	1.22 (1.02 to 1.46)	-367 (-196 to -2750)
Cardiovascular		
Unexpected	1.14 (0.84 to 1.56)	-1699 (-510 to 1278)
Myocardial infarction	1.67 (0.80 to 3.46)	-2337 (-969 to 5674)
Congestive heart failure	1.30 (0.72 to 2.36)	-3115 (-957 to 2481)
Procedure related	2.20 (0.75 to 6.47)	-3115 (-1336 to 9380)
Arrhythmia	0.33 (0.10 to 1.06)	2337 (1169 to 2359681)
Stroke	0.75 (0.31 to 0.69)	6231 (-3037 to 1538)

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Mechanism of cell death induced by arsenic trioxide in non–small-cell lung carcinoma and mesothelioma cell lines

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Introduction: Arsenic trioxide (ATO) has been proven to be an effective treatment in acute promyelocytic leukaemia and its activity has also been noted in some other solid tumours. The objectives of this study were to examine the in-vitro effects of ATO in cell proliferation and the molecular mechanisms underlying the ATO-induced cellular responses in non–small-cell lung carcinoma (NSCLC) and mesothelioma cell lines.

Methods: Seven NSCLC cell lines and five mesothelioma cell lines were employed. Effects of ATO treatment were studied to investigate cell viability, phosphatidylserine (PS) externalisation and mitochondrial membrane depolarisation. Expression of apoptotic-related proteins and thymidylate synthase (TS) were studied by Western blotting.

Results: After incubation with ATO for 72 hours, cellular growth was inhibited in all cell lines with IC50 values below 8 micromolar (within clinically reachable concentration). Only H23 cells (a NSCLC) treated with ATO displayed PS externalisation. All ATO-treated cell lines underwent mitochondrial membrane depolarisation. From our preliminary results, Bcl-2 (anti-apoptotic factor) was down-regulated in all ATO-treated cell lines. Truncation of BID (apoptotic factor) and PARP cleavage (apoptotic factor) were demonstrated in 3 and 4 of the NSCLC cell lines respectively. ATO could also down-regulate TS (DNA synthesis) expression in most cell lines with demonstrable basal TS expression.

Conclusion: ATO can induce apoptosis (through mitochondrial pathway) and TS down-regulation (for those with basal TS expression) in NSCLC and mesothelioma cell lines at clinically achievable concentrations. The mechanisms and in-vivo studies for treatment of NSCLC and mesothelioma will be further explored.

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Non-dysplastic polyps and their association with synchronous colonic neoplasm

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Background: Non-dysplastic polyp includes both serrated polyps (sessile serrated polyp and serrated adenoma) and hyperplastic polyps. Serrated polyps can progress into colorectal cancer through an alternate serrated pathway and recent data showed that they are associated with synchronous advanced colonic neoplasm. Although small hyperplastic polyps are generally considered to have low risk of cancer progression, the significance of large (>5 mm) hyperplastic polyp remains unclear.

Objective: To characterise patients with serrated polyps and large hyperplastic polyps and their association with advanced colonic neoplasm.

Methods: All patients who had undergone colonoscopy and polypectomy in Queen Mary Hospital between January 2008 and June 2010 were retrieved. Histology reports were reviewed and those with large hyperplastic polyp (>5 mm) and serrated polyp were included. Demographic parameters, polyp location and its association with colonic adenoma and colorectal cancer were evaluated.

Results: Of 2091 patients who underwent colonoscopy and polypectomy, 206 (9.8%) patients with non-dysplastic polyps were identified (47 with serrated polyps and 159 with large hyperplastic polyps). The median age was 65 years (64.6% male). Twenty (42.6%) patients with serrated polyps and 74 (46.5%) patients with large hyperplastic polyps had non-dysplastic polyps located in proximal colon. When compared to patients with serrated polyp, the percentage of different synchronous colonic neoplasm in patients with large hyperplastic polyps are: adenoma 31.4% vs 42.6%, P=0.158; advanced neoplasm: 12.6% vs 12.8%, P=0.973; and cancer 5.0% vs 8.5%, P=0.476.

Conclusion: A large proportion of non-dysplastic polyps are located in the proximal colon, which is not reached by flexible sigmoidoscopy. Synchronous colonic neoplasm and colorectal cancer are commonly seen in patients with serrated polyp and large hyperplastic polyps.

Clinical and neuroradiological features of viral encephalitis: a hospital-based study

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Introduction: Viral encephalitis is an important infection of the central nervous system with significant morbidity and mortality. We studied the clinical, laboratory and radiological characteristics of viral encephalitis patients managed in our hospital, focusing on factors associated with poor prognosis.

Methods: We retrospectively studied the clinical features of patients diagnosed with viral encephalitis and managed at a regional hospital in Hong Kong during 2000-2010. Patients with a poor prognosis were defined as death, Rankin test score ≥3 or Modified Barthel Index ≤60 at 6 months after admission.

Results: A total of 43 patients with viral encephalitis were identified. The mean age was 52 years with 49% males. The aetiologies of viral encephalitis were identified in 33% of cases (herpes simplex virus 26%, varicella zoster virus 5%, and Japanese B virus 2%). 21% of patients had a poor prognosis at 6 months after admission. Patients of older age, with a known history of hypertension, presenting with focal neurological signs and requiring prolonged hospitalisation were associated with poor prognosis ($P<0.05$). A haemorrhagic lesion on cranial MRI was also associated with a poor prognosis and was identified as an independent poor prognostic indicator in multivariate logistic regression analysis ($P<0.05$).

Conclusion: Herpes simplex virus is the most common cause of viral encephalitis in our locality. 22% of patients are associated with a poor outcome and presence of a haemorrhagic lesion on cranial MRI is an independent poor prognostic indicator.

The role of circulating serotonin in the development of chronic obstructive pulmonary disease

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Background: Cigarette smoking is a major risk factor in the development of age-related chronic obstructive pulmonary disease (COPD). The degree of cigarette smoking mediated the association between serotonin transporter (SERT) gene polymorphism and COPD. However, the interrelation between circulating 5-HT, cigarette smoking and COPD is mainly unknown. The current study aimed to investigate the mediation effects of 5-HT on cigarette smoking-induced COPD and the relation between plasma 5-HT and age.

Methods: The association between plasma 5-HT, age and COPD was analysed in a total of 62 COPD patients and 117 control subjects. Passive smoking rat model was also used to further explore the effect of cigarette smoking on plasma and bronchoalveolar lavage (BAL) 5-HT levels.

Results: Elevated plasma 5-HT levels were associated with the risk in developing COPD (OR=1.221; 95% CI, 1.123-1.319; $P<0.0001$). The effect remained significant after adjustment of age and pack-years smoked. Furthermore, plasma 5-HT was found to mediate the relation between pack-years smoked and COPD. Positive correlation (r=0.303; 95% CI, 0.057-0.514; $P=0.017$) was found between plasma 5-HT and age in COPD but not in control groups. In support, passive smoking significantly elevated plasma 5-HT levels and showed a trend of increase in BAL 5-HT levels in the rat model in vivo.

Conclusion: Our results demonstrate the mediation role of plasma 5-HT in cigarette smoking-induced COPD. Smoking exposure for a limited period of time was associated with high 5-HT level. Further studies will be required to elucidate the mechanism of CS on the regulation of 5-HT.

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Brainstem encephalitis in neuromyelitis optica spectrum disorders

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Introduction: Neuromyelitis optica spectrum disorders (NMOSD) are severe CNS inflammatory demyelinating disorders characterised by extensive acute myelitis (AM) and optic neuritis (ON). NMOSD are probably due to aquaporin-4 (AQP4) autoimmunity as ~60-80% NMO patients are seropositive for AQP4 autoantibodies (AQP4 Ab). We recently reported that brain involvement is common in NMOSD in Hong Kong Chinese, and the brainstem is the most common site of brain involvement, which clinically presents as brainstem encephalitis (BE).

Aim: To study the clinical and radiological features of BE in local NMOSD patients.

Methods: NMOSD patients diagnosed (according to Wingerchuk's criteria) and followed up in QMH from January 1988 to September 2011 were reviewed. The clinical and neuroradiological characteristics of those with brainstem encephalitis were studied in details. AQP4 Ab was assayed by cell-based immunofluorescence using transfected HEK293 cells expressing full length human AQP4 gene.

Results: Forty NMOSD patients (21 NMO, 11 relapsing AM, 6 relapsing ON, 1 relapsing BE, 1 AM with BE) were studied. Their mean onset age was 39.5 years (range, 17-80), 36 (90%) were female; mean follow-up duration 5.0 years (range, 1-16). AQP4 Ab were detected in 25 (62.5%). Twelve (30%) of the 40 patients had clinical attacks of BE presenting with diplopia, dizziness, ataxia, vertigo, nausea, vomiting, third nerve palsy, internuclear ophthalmoplegia, facial weakness (both LMN and UMN type), tinnitis, hearing loss, aphonia, dysphagia, aspiration pneumonia, autonomic dysfunction, facial sensory loss, long-tract sign. MRI brain revealed lesions in midbrain (10%), pons (10%) and medulla (20%), with brainstem lesion continuous with extensive cervical myelitis as the most common brainstem lesion in patients with BE. Two patients succumbed during severe BE involving medulla in continuity with extensive cervical myelitis.

Conclusion: Brainstem encephalitis is common in NMOSD among Hong Kong Chinese, which present with diverse bulbar symptoms and signs. The medulla is the most frequently affected site.

Serum adiponectin levels are predictive of carotid intima-medial thickness in a 5-year community-based prospective study

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Introduction: Hypoadiponectinaemia has been shown to predict the development of type 2 diabetes, hypertension, and myocardial infarction in prospective studies. We have previously reported that hypoadiponectinaemia is associated with impaired endothelium-dependent vasodilation both in healthy controls and diabetic subjects. In this community-based prospective cohort study, we examined the predictive value of serum adiponectin levels on carotid intima-medial thickness, a marker of atherosclerotic disease, in Hong Kong Chinese.

Methods: Subjects were recruited from the Hong Kong Cardiovascular Risk Factors Prevalence Study 2 (CRISPS 2) cohort. Those with known cardiovascular disease(s) were excluded. Fasting plasma glucose, insulin, and lipid profile were measured. Baseline serum levels of adiponectin and carotid intima-media thickness (IMT) were determined. Subjects were followed prospectively for 5 years.

Results: 265 subjects (129 male, 54.6 ± 12.3 years) were included in the final analysis. At baseline, 86 and 34 subjects have hypertension and type 2 diabetes respectively. Their body mass index (BMI) was 24.9 ± 3.7 kg/m^2, systolic blood pressure 125 ± 20 mm Hg, diastolic blood pressure 76 ± 11 mm Hg, LDL-cholesterol 3.4 ± 0.8 mmol/L, HDL-cholesterol 1.3 ± 0.4 mmol/L, fasting glucose 5.3 ± 1.2 mmol/L, HOMA-IR median (interquartile range) 1.80 (1.28-2.76), and adiponectin 5.28 mg/L [3.29-7.93]. Over 5 years, carotid IMT increased from 0.62 mm (0.52-0.73) to 0.67 (0.57-0.78) [P<0.001, paired *t*-test]. Linear regression analysis showed that age (β=0.22, P=0.02), smoking status (β=0.16, P=0.014), baseline IMT (β=0.59, P<0.001) and sex-specific median adiponectin (β= –0.14, P=0.031) were independent predictors of IMT at 5 years in men, after controlling for the conventional cardiovascular risk factors. Adiponectin (sex-specific median) was not significantly predictive of 5-year IMT in women.

Conclusion: In this 5-year prospective study, hypoadiponectinaemia was an independent predictor of IMT thickening in Hong Kong Chinese men. Our data suggest that the measurement of serum adiponectin would allow for early risk stratification even in a community-based cohort, and enable timely implementation of medical interventions to reduce future cardiovascular events among subjects with hypoadiponectinaemia.

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Evaluation of neointimal healing of endothelial progenitor cell capturing sirolimus-eluting (COMBO) stent by optical coherence tomography: the EGO-COMBO pilot study (interim results)

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Background: COMBO stent (OrbusNeich Medical, FL, US) is a hybrid version of the endothelial progenitor cell (EPC) capturing Genous stent (OrbusNeich), with an additional abluminal 5 µg/mm sirolimus coating, about ½ dose of Cypher stent but with a similar release profile (about 90% of drug released at 35 days) via a Surmodics SynBiosys bio-degradable polymer, aiming at optimal neointimal suppression similar to other DES while retaining the EPC capturing benefit (in theory better endothelialisation and less late stent thrombosis from non-healing) as reported in animal model. Such combined benefits were evaluated clinically in this study.

Methods: In this prospective, single centre, pilot study, 60 patients treated by COMBO stent were randomised to four monthly groups. Frequency domain optical coherence tomography (FD-OCT) was performed sequentially at baseline post-stenting, at early follow-ups (for early neointimal healing in 4 groups at 2nd, 3rd, 4th, and 5th month), and at 9 months (for OCT late loss). Independent OCT core laboratory performed the covered strut % and neointima analyses, while in-house analysis further stratified the early strut coverage into six categories (A to F).

Results: To date, all 60 patients (30% diabetic, 87 COMBO stents implanted) were enrolled and 40 had the first OCT restudy. A total of 7004 frames and 60 069 struts were analysed. The mean percentages of covered struts (with properly apposition) were 74.4%, 84.0%, 87.4%, and 95.6%, P=0.014, 0.226, and 0.046, between 2nd to 5th month, respectively. No MACE was recorded up till this abstract. Study limitations were: (1) no other DES control arm, and (2) OCT classification of early strut coverage requires further validation.

Interim Conclusion: For the first time, the healing profile (strut coverage) of a new EPC capturing DES (COMBO stent) was established; near 100% stent coverage was recorded by OCT in 140 days.

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9-Month optical coherence tomography results comparing COMBO to TAXUS DES in a subset of the REMEDEE Study

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Background: The REMEDEE Study is a multi-centre, randomised (2:1) study comparing 9-month angiographic late loss between the COMBO stent (OrbusNeich Medical, FL, US) and the TAXUS placitaxol DES (Boston Scientific, US). The COMBO stent is a hybrid version of the endothelial progenitor cell (EPC) capturing Genous stent (OrbusNeich Medical) with an additional abluminal, 5 µg/mm sirolimus coating, about ½ the dose of Cypher stent (Johnson & Johnson, US) but with a similar release profile (about 90% of drug released at 35 days) via a Surmodics SynBiosys bio-degradable polymer, aiming at achieving neointima suppression similar to other DES while retaining the EPC capturing benefits (in theory better neointimal healing and less late stent thrombosis) as already reported in animal model. We report the 9-month optical coherence tomography (OCT) differences observed, especially on in-stent late loss, between these two types of stents in our patients.

Methods: A total of 31 patients (COMBO 20, TAXUS 11) were enrolled in the REMEDEE Study in this centre. During the 9-month angiographic restudy, OCT was performed in the last 27 consecutive patients (COMBO 17, TAXUS 10) after the availability of the LightLab C7XR OCT machine ((LightLab Imaging, MA, US). Minimal and maximal neointimal thickness (NIT) and neointimal area (NIA) per OCT frame were analysed and compared. Strut coverage was recorded according to our neointimal coverage classification into six categories.

Results: Baseline clinical and intervention characteristics were similar except more patients had hyperlipidaemia in the COMBO group (59.3% vs 22.2%, P=0.028). 29.6% of patients had diabetes. Comparing the COMBO to the TAXUS group, 18 vs 10 stents were implanted; a total of 1624 OCT frames (15 121 struts) vs 1136 frames (11 479 struts) were analysed; mean minimal NIT was 0.054 ± 0.043 mm vs 0.056 ± 0.060 mm, P=0.227, and mean maximal NIT was 0.250 ± 0.010 mm vs 0.306 ± 0.167 mm, P<0.001, 95% CI (0.044-0.066), effect size=0.43; and mean NIA was 1.187 ± 0.506 mm^2 vs 1.379 ± 0.714 mm^2, P<0.001, 95% CI (0.144-0.241), effect size=0.32. In OCT frames with ≥4 struts and not over side branches (COMBO 1560 and TAXUS 1094 frames), mean stent strut coverage were 99.2 ± 3.8% vs 98.4 ± 5.5%, P<0.001.

Conclusion: The COMBO hybrid stent behaved like a DES with low 9-month NIT and NIA as assessed by OCT; both parameters were significantly better than the TAXUS stent. The near 100% strut coverage at 9 months probably supported that the abluminal sirolimus coating was not annihilative to the pro-healing benefits of the EPC coating.

45 Evaluation of early healing of endothelial progenitor cell capturing (GENOUS) stent by optical coherence tomography: the EGO Study (interim results)

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Background: Autopsy reports suggest several months are required for bare metal stent (BMS) to become fully endothelialised. Development of neointimal coverage shortly after stenting (around first month) has never been established in-vivo. This becomes possible with the ultra-high resolution (10 μm) of frequency domain optical coherence tomography (FD-OCT). Genous Stent (OrbusNeich Medical, FL, US), with animal models supporting its pro-healing benefits via circulatory endothelial progenitor cell (EPC) capturing, should exhibit earlier neointimal healing than other BMS, and therefore was studied.

Methods: Fifty patients successfully treated with Genous Stent were prospectively randomised to 5-weekly groups for early OCT restudy. Most patients received primary PCI for ACS and had remaining diseases indicated for a second (staged) PCI, when the OCT restudy was performed. Independent OCT core laboratory blinded from the clinical data performed the covered strut % and neointima analyses, while in-house analyses further classified the strut coverage into six categories (A to F); malapposed struts and struts over side branches were analysed separately.

Results: To date, 32 patients (ACS 61.3%, diabetes 41.9%) were enrolled and 31 had OCT restudy. A total of 4621 OCT frames and 46 769 struts were analysed. The mean percentages of covered struts (with proper apposition) were 46.5%, 79.9%, 87.1%, 92.6% and 95.8%, between group P values <0.001, 0.027, 0.076, and 0.320, from 2nd to 6th week, respectively.

Interim Conclusion: For the first time, the healing profile (neointimal coverage) of EPC capturing BMS was established. Near 100% stent coverage was recorded in 6 weeks.

46 The combined use of phenol and botulinum toxin type A injection to promote gait training in chronic stroke survivors with spasticity: a pilot randomised, double-blind, placebo-controlled study

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Introduction: This study aimed to determine whether the treatment of spasticity by phenol and/or botulinum toxin type A injection could enhance the training effect obtained from gait training in chronic stroke patients with motor function limited by spasticity.

Methods: Twelve patients with stroke for at least more than 6 months having persistent lower limb spasticity were recruited. They were randomly assigned into two groups: (1) Treatment group: phenol motor point block and/or botulinum toxin type A injection; (2) Placebo group: normal saline injection; both followed by 8 weeks of gait training. Main outcome measures were (1) gait velocity, (2) Six-Minute Walk Test, and (3) spasticity measurement for the most affected joint in lower limbs: the modified Modified Ashworth Scale. These were measured at baseline and 2 months.

Results: There was statistically significant improvement in gait velocity within the treatment group from 1.5 km/h (interquartile range, 1.05-2.23 km/h) at baseline to 1.95 km/h (interquartile range, 1.38-2.48 km/h) at 2 months (P=0.046) but not in the control group. Average Six-Minute Walk distance in the treatment group improved from 148.1 m to 199.7 m but not reaching statistically significant level. Spasticity reduced from MMAS score of 2 to 1.5 after active treatment, though again statistically non-significant. There was a trend difference between the treatment and control groups regarding improvement in gait velocity (0.4 km/h vs 0.3 km/h, P=0.575). However, there were no significant differences between the treatment and placebo groups in gait velocity and other outcome measures.

Conclusion: Participants who received 8 weeks of gait training in the active spasticity treatment group showed statistically significant improvement in gait velocity. Comparing active and control groups, a trend difference of improvement with additional use of phenol motor point block and/or botulinum toxin type A injection was observed. Further researches using larger samples were warranted to confirm the real benefit of this combined approach of rehabilitation in chronic stroke patients.

Cryptococcal meningitis: a hospital-based study

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Objective: Cryptococcus meningitis (CM) is an uncommon CNS infection. This study aimed to find out the clinical and radiological characteristics of CM patients.

Methods: Records of CM patients diagnosed and treated in Queen Mary Hospital during the period 1995-2008 were retrospectively studied. Cryptococcus meningitis was diagnosed by raised cryptococcal antigen titer in CSF with exclusion of other causes of meningitis. Patients who died from CM or who had modified Barthel Index (mBI) <12 at 6 months after initiation of anti-fugal therapy was classified as having poor clinical outcome.

Results: Twenty-one CM patients were identified, 11 were male. Fourteen (67%) patients had medical comorbidities at presentation and were immunocompromised: six (29%) had autoimmune diseases such as SLE on immunosuppressants, four (19%) were HIV carriers, two (10%) had chronic rheumatic heart disease, and two had renal transplant on immunosuppressants. The mean age of onset was 48 years (SD, 15). The mean duration of presenting symptoms was 33 days (SD, 37 days). Presenting symptoms included headache (76%), fever (71%), neurological deficits (43%, including incoordination, limb weakness, diplopia, blurring of vision), confusion (33%), lethargy/dizziness (28%), nausea/vomiting (28%), neck pain (24%), decreased GC (5%), personality change (5%) and incontinence (5%). The most common clinical signs at presentation were neck stiffness (33%) and cranial nerves palsy (24%). The mean opening pressure on LP was 21 cm water (range, 3-55; SD, 13), mean CSF cell count 61/μL (range, 2-290/μL; SD, 99/μL), mean CSF glucose 2.2 mmol/L (range, 0.9-3.8; SD, 0.9) and mean CSF protein 1.4 g/L (range, 0.5-6; SD, 1.4). Initial neuroimaging revealed abnormalities in seven (33%) patients, including leptomeningeal enhancement (1), calcification from previous inflammation (1), hydrocephalus (1), cerebral oedema (1), and cerebral infarctions (3). Standard anti-fungal medications was started immediately once CM was diagnosed. Five (24%) patients succumbed from CM despite active treatment, including three HIV carriers, one ESRF patient post-renal transplantation on immunosuppressants, and an 83-year-old man with good past health. Ten (48%) patients had good recovery with mBI at 6 months >90, and only one of the 16 survivors had poor outcome with mBI at 6 months <12.

Conclusion: Cryptococcus meningitis is a serious CNS infection predominantly affecting immunocompromised patients with a mortality rate of 24%. HIV infection, immunosuppression for organ transplantation and advanced age might be risk factors for mortality from CM. Most survivors had satisfactory functional recovery with only mild or minimal neurological disability.

Neoadjuvant systemic treatment followed by breast conserving surgery is effective in Asian Stage II and III breast cancer patients

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Background: Neoadjuvant systemic treatment aims to reduce primary tumour size and facilitate curative resection in non-metastatic breast cancer. In Asian patients planned for breast conserving surgery (BCS), small breast volume leads to distinct oncological and cosmetic considerations.

Methods: We evaluated a retrospective cohort of Asian female stage II to III breast cancer patients treated with neoadjuvant systemic therapy from June 2004 to July 2010. The patients were divided based on initial tumour stage into subgroups: (i) T1-2 and (ii) T3-4. Pathological complete response (pCR) rates, BCS rates and change in median Ki67 indices in patients with residual tumour were analysed. In an exploratory analysis, patients treated from July 2008-2010 ,where complete pre-treatment staging and immunohistochemistry (IHC) markers were available, were classified into Luminal A (Lum A), B (Lum B), HER2+ and Triple negative (TN) subgroups according to their IHC markers and Ki67 percentage. The Ki67 cutoff for Lum A versus Lum B was set at 12%.

Results: A total of 78 patients were included in the overall analysis; 40 (51.3%) had T1-2 while 38 (48.7%) had T3-4 disease before treatment. Neoadjuvant treatment regimens used included taxane-based (59%), anthracycline-based (2.6%), both taxane- and anthracycline-containing (17.9%), and others. In the T1-2 group, a median of six treatment cycles were given. Five (12.5%) patients achieved pCR and 14 (35%) underwent BCS. The median Ki67 reduced by 67% (P<0.0005). The patients in the T3-4 group were also treated with a median of 6 cycles; pCR and BCS rates were 13.2% and 8% respectively. The median Ki67 reduced by 59% (P=0.005). The biological classification analysis contained 39 patients, Lum A 10% (n=4), Lum B 36% (n=16), Her2 38% (n=15), and TN 15% (n=6). All patients received a taxane-based neoadjvuant regimen and all HER2 + patients received herceptin in addition. Primary tumor pCR rates according to subtypes were LumA 0%, LumB 7%, HER2+ 47%, and TN 17%. BCS rates were Lum A 25%, Lum B 36%, HER2+ 20%, and TN 33%.

Conclusion: Neoadjuvant treatment followed by BCS is an effective treatment strategy in Asian stage II and III breast cancer especially for initial T1-2 patients. In larger tumours, despite response to neoadjuvant treatment, increased tumour-to-breast size ratio remains an obstacle to BCS. The pCR rates in our cohort of patients are comparable to published international datasets. In particular, HER2+ tumours showed excellent pCR rates and this subgroup of patients may derive the most benefit from neoadjuvant chemobiological therapy prior to BCS.

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Is human cytomegalovirus infection associated with hypertension? The United States National Health and Nutrition Examination Survey 1999–2002

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Introduction: Human cytomegalovirus (HCMV) infection is common and is thought to play a part in cardiovascular disease. Recent studies suggested a link between HCMV infection and hypertension. Therefore we studied the association between HCMV and hypertension in the United States National Health and Nutrition Examination Survey 1999-2002.

Methods: We analysed data on 3022 men and 3393 women in the survey. We included participants aged 16 to 49 years who had valid data on HCMV infection and hypertension.

Results: Of the participants, 54.9% had HCMV infection and 15.9% had hypertension. There were ethnic differences in HCMV infection (P<0.001) and hypertension (P<0.001). Before adjustment, HCMV infection was strongly associated with hypertension in women (OR=1.60; 95% CI, 1.18-2.17; P=0.004) but not in men. After adjustment for race/ethnicity, the association between HCMV infection and hypertension in women remained significant (OR=1.51; 95% CI, 1.10-2.06; P=0.012). Further adjustment for BMI, diabetes status and hypercholesterolemia attenuated the association (OR=1.41; 95% CI, 1.02-1.95; P=0.038). However, after adjusting for age, the association was no longer significant (OR=1.20; 95% CI, 0.84-1.71; P=0.30).

Conclusions: In this large population-based survey, there is no strong evidence that HCMV is a significant cause of hypertension. Hypertension in women may be associated with HCMV, but this could be explained at least partly by age. Further studies are needed before definite conclusions can be drawn.

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Endothelium-selective activation of AMP-activated protein kinase alleviates diabetes-induced endothelial dysfunction by enhancing reendothelialisation and endothelial progenitor cell mobilisation in mice

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Introduction: Vascular dysfunction is commonly observed in diabetic patients. Endothelial injury can be repaired in part by endothelial progenitor cells (EPCs). In diabetes, impaired functionality and reduced number of EPCs are reported. AMP-activated kinase (AMPK) is a well-known target of several anti-diabetic and cardiovascular drugs. The objective of this study was to examine whether the activation of AMPK in endothelium alone is sufficient to prevent diabetes-induced impairment in vascular function, possibly by improving EPC function and endothelial repair using a tissue-specific transgenic mouse model.

Methods: Transgenic mice with endothelium-selective expression of a constitutively-active AMPK (AMPK-Tg) were generated using the T-cadherin gene promoter. Type 1 diabetes was induced by i.p. injection with streptozotocin. Wire-mediated injury was introduced to the right common carotid artery of both WT and AMPK-Tg mice. Vascular relaxation to the α2-adrenergic receptor agonist UK14304 was assessed using a wire myograph. Vascular repair, or reendothelialisation, was examined using Evans blue staining. Circulating EPC numbers were quantified by flow cytometry analysis. Bone marrow-derived EPCs (BM-EPCs) were subjected to high glucose (25 mM) stimulation and expression of the antioxidant protein heme oxygenase (HO)-1 and mobilisation regulator stromal cell derived factor (SDF)-1, together with generation of reactive oxygen species (ROS), were studied.

Results: (1) Dose-dependent relaxation to UK14304 in injured carotid arteries was augmented in AMPK-Tg mice compared to WT mice in both healthy (pEC50 = 5.3 ± 0.1 vs 4.4 ± 0.6) and diabetic (pEC50 = 5.9 ± 0.1 vs 3.9 ± 0.1) conditions. (2) Reendothelialisation of carotid injury was improved in diabetic AMPK-Tg vs diabetic WT mice (147.2 ± 7.9% vs 95.6 ± 3.5%). (3) concomitant with elevated levels of circulating EPCs (AMPK-Tg vs WT mice = 0.15 ± 0.02% vs 0.06 ± 0.02%), indicating enhanced EPC mobilisation. (4) Mechanistic studies revealed an elevated level of SDF-1 in cultured BM-EPCs from AMPK-Tg compared to WT mice, in addition to a higher level of expression of HO-1 (the upstream regulator of SDF-1). (5) Further studies demonstrated a diminished production of ROS in AMPK-Tg BM-EPCs, showing a relationship between suppressed oxidative stress and the increased antioxidant protein expression, and higher SDF-1 levels in AMPK-Tg mice.

Conclusion: The improvement in vascular reactivity in diabetic AMPK-Tg mice could be attributed to the accelerated vascular repair and improved EPC mobilisation upon endothelial activation of AMPK. Concurrently, a lower level of oxidative stress and increased expression of HO-1 and SDF-1 may provide mechanistic explanation for the improvement in EPC function. Activation of AMPK may represent an attractive therapeutic strategy for cardiovascular disease in diabetes.

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Anti-inflammatory effects of bone morphogenetic protein 7 on advanced glycation end products–induced tubular injury

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Introduction: Formation and accumulation of advanced glycation end products (AGEs) are remarkably accelerated in the diabetic kidney. We recently demonstrated that AGEs caused various pro-inflammatory and pro-fibrotic responses in human proximal tubular epithelial cells (PTEC). Bone morphogenetic protein (BMP) 7 has been reported to have renoprotective effects in a variety of cell types and disease models; however, data are lacking in AGEs-induced renal tubular inflammation. Therefore, this study aimed to explore the therapeutic effects of BMP7 on AGEs-induced tubular injury and the possible mechanisms.

Methods: Primary human proximal tubular epithelial cells were growth-arrested and exposed to glycated human serum albumin (AGE-HSA) with or without recombinant human BMP7 (rhBMP7). Pro-inflammatory chemokines and cytokines were detected at both gene and protein levels by real-time PCR and ELISA, respectively. Inhibitors of different signalling pathways were used (SB203580, PD98059 and PDTC for p38 MAPK, p44/42 MAPK and NF-κB respectively) to study the involvement of these pathways.

Results: AGE-HSA (100 μg/mL) induced PTEC expression of IL8 through both p38 and p44/42 MAPK pathways, sICAM-1 through p44/42, MCP1 through p38 MAPK, and IL6 through activation of NF-κB. BMP7 (5-200 ng/mL) dose-dependently attenuated the expression sICAM-1, MCP1, IL8 and IL6 at both mRNA and protein levels. Moreover, BMP7 suppressed phosphorylation of p38, p44/42 and I-kB which was induced by AGE-HSA.

Conclusion: Our results demonstrated in vitro that BMP7 can attenuate pro-inflammatory responses to AGE stimulation in PTEC via suppression of p38 MAPK, p44/42 and NF-κB signalling pathways.

Sarco/endoplasmic reticulum Ca-ATPase (SERCA) pump is a more effective calcium-handling mediator than sodium-calcium exchanger (NCX) in human embryonic stem cell–derived ventricular cardiomyocytes

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Introduction: Ventricular (V) cardiomyocytes (CMs) are non-regenerative. Self-renewable pluripotent human embryonic stem cells (hESCs) can differentiate into CMs for cell-based therapies. We have previously shown that hESC-derived CMs display immature Ca-handling properties, with smaller transient amplitudes and slower upstroke and decay kinetics. These functional immaturities can be attributed to their proteomic differences in crucial Ca-handling proteins such as the complete absence of triadin, junctin, CSQ, phospholamban. Indeed, forced CSQ expression partially matures Ca transient properties. During diastole, sarco/endoplasmic reticulum Ca-ATPase (SERCA) and sodium-calcium exchanger (NCX) sequester and extrude Ca ions, respectively, after the transient peak to return cytosolic Ca to the resting level. We have reported that NCX, robustly expressed in hESC-VCMs but much less so in the adult counterparts (>10-fold), is a functional determinant of immature Ca homeostasis. Unlike NCX, however, SERCA is comparably expressed in hESC- and adult-VCMs.

Methods: Ventricular cardiomyocytes are differentiated from hESCs by directed differentiation protocal, then they are labelled with LV-MLC2v-tdTomato-t2A-zeo for selection. Confocal imaging in 2D mode and line-scan mode are used to capture Ca^{2+} transient and sparks, respectively.

Results: we found that shRNA-based suppression of NCX in hESC-VCMs (to a level similar to adult NCX, and therefore a higher SERCA/NCX ratio than control) similarly led to reduced amplitudes and slowed kinetics of both caffeine- and electrically-induced Ca transients (by ~2-3-fold). By contrast, SERCA overexpression (to similarly increase SERCA/NCX) produced an opposite chronotropic effect by augmenting the same parameters (by~2-fold).

Conclusion: We conclude that SERCA pump is a more effective Ca-handling mediator than the NCX to target in hESC-derived VCMs for inducing positive chronotropic effects.

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53 Association of depressive symptoms with disease activity, functional status and quality of life in Chinese patients with rheumatoid arthritis

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Introduction: Depression has previously been reported to be more common among patients with rheumatoid arthritis (RA). Whether active disease and decreased function are associated with depressive symptoms in Chinese patients with RA remains controversial. The study aimed to examine whether disease activity and functional status were associated with depressive symptoms in a cohort of Chinese RA patients and their effects on the health-related quality of life (QOL).

Methods: Consecutive RA patients were recruited from a local rheumatology clinic. Socio-demographics, depressive symptoms (depression subscale of the Hospital Anxiety and Depression Scale [HADS]), disease activity (Disease Activity Score using 28 joint counts [DAS28]), functional status (Health Assessment Questionnaire [HAQ], and functional class) and QOL (Short-Form 36 version 2 health survey [SF36v2]) of the patients were analysed.

Results: A total of 202 RA patients were recruited. Multivariate linear regression using HADS depression score as dependent variable showed that higher HAQ scores (β=0.33, P<0.001), higher functional class (β=0.41, P<0.001) and past history of depression (β=0.17, P<0.001) were significantly associated with depressive symptoms. DAS28 was not found to be a significant factor. The RA cohort was found to have worse QOL compared with the general population in Hong Kong. The HADS depression score, DAS28 and HAQ were all inversely correlated with SF36v2 physical and mental health with P<0.001.

Conclusion: Functional limitation and past history of depression were independently associated with depressive symptoms in RA patients. Both disease parameters including DAS28 and HAQ as well as depressive symptoms contributed to poor QOL of these patients.

54 Age-dependent alterations of cigarette smoke-induced oxidative and inflammatory responses in rats

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Background: Cigarette smoking (CS) is a leading cause of chronic obstructive pulmonary disease (COPD). The prevalence of COPD is much higher among the elderly. However, the impact of early-age CS exposure on CS-induced COPD is unknown. This study aimed to investigate whether CS-induced oxidative and inflammatory responses were age-dependent in an acute CS-exposed rat model.

Methods: Male Sprague-Dawley rats of 6-7 weeks of age (juvenile) and >8 months of age (adult) were randomly divided into two groups, respectively (n=5-6 in each group), one of which was exposed to 4% CS for 1 hour twice daily for 5 days in ventilated smoking chambers, while the other group was exposed to sham air (SA). Blood and lung tissues were collected 24 hours after last CS exposure. Oxidative stress markers such as 8-isoprostane and malondialdehyde (MDA) and pro-/anti-inflammatory markers such as transforming growth factor-β_1 (TGF-β_1) and adiponectin were measured.

Results: Cigarette smoking exposure significantly elevated serum 8-isoprostane and lung MDA levels in juvenile group (8-isoprostane: 4.56 ± 0.33 ng/mL vs 2.46 ± 0.21 ng/mL for CS- and SA-exposed rats respectively; P<0.01) [MDA: 12.06 ± 0.94 nmol/mg protein vs 6.06 ± 0.35 nmol/mg protein for CS- and SA-exposed rats respectively; P<0.001) but not in adult group. In contrast, serum adiponectin level was unaltered in juvenile group but significantly decreased in adult group after CS exposure (9.72 ± 0.65 μg/mL vs 14.00 ± 0.9 μg/mL for CS- and SA-exposed rats respectively; P<0.01). For plasma TGF-β_1 level, CS exposure caused significant elevation in adult group (15.70 ± 3.20 ng/mL vs 8.24 ± 1.33 ng/mL for CS- and SA-exposed rats respectively; P<0.05) but not in juvenile group.

Conclusion: The oxidative and inflammatory responses to CS vary depending on age. The early life is particularly at risk for the development of CS-induced oxidative stress, which may be one of the mechanisms leading to lung tissue damage following CS exposure over time.

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The role of autophagy induced by erlotinib treatment in non–small-cell lung carcinoma

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Introduction: Non–small-cell lung cancer NSCLC is one of the leading causes of cancer deaths worldwide. Epidermal growth factor receptor (EGFR) is frequently deregulated in NSCLC, which allows specific tyrosine kinase inhibitor (TKI) treatment as exemplified by erlotinib. However, the efficacy of erlotinib is significantly limited by primary and acquired resistance. Previous reports have suggested a possible association between TKI treatment and autophagy induction in other cancers. This study aimed to investigate if autophagy can serve as a drug resistance mechanism upon erlotinib treatment in NSCLC.

Methods: Three NSCLC cell lines with different EGFR mutation status and different sensitivity to erlotinib were chosen in our study. In order to determine cell proliferation and cell death upon treatment, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (proliferation), flow cytometry (apoptosis), and Western-blot for apoptotic proteins were performed. As for detection of autophagy induction, microtubule associated protein Light Chain 3 (LC3) was detected by Western-blot, and acridine orange staining fluorescence microscopy was conducted to detect the formation of acidic vesicular organelles (AVO).

Results: After 72 hours of erlotinib treatment, our MTT data showed that HCC4006 was sensitive (IC50:0.32uM±0.1), H358 was intermediate (IC 50:7.4uM±2.9), while H23 was resistant (IC 50:12.5uM±2.4). Western blot showed that cleaved caspase 3 and cleaved PARP increased in HCC 4006 and H358 cell lines after erlotinib treatment, but not in H23. Flow cytometry with annexin-V PE staining showed similar pattern among these three cell lines, in line with their drug sensitivity. Erlotinib treatment could increase both the LC3II expression levels and AVO positive cells in HCC4006 (sensitive cell line), but not in H358 and H23. In HCC4006, the combination of erlotinib with autophagy inhibitor (chloroquine) caused significantly more cell death (49.3% ± 4.8) compared with single treatments (erlotinib 9.4% ± 2.4) or chloroquine (9.0% ± 1.8). In cell lines without induced autophagy (H358 and H23), the combination of chloroquine with erlotinib was similar to single drug treatment.

Conclusions: Erlotinib can induce both apoptosis and autophagy in sensitive cell line HCC4006. Inhibition of autophagy with chloroquine in HCC4006 can enhance cell death induced by erlotinib treatment. We conclude that autophagy is induced by erlotinib in sensitive cells lines as a protective mechanism, and the inhibition of autophagy can potentially enhance the effect of erlotinib.

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 (hESCs) 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 (IK1), among the panoply of sarcolemmal ionic currents investigated (INa+/ICaL+/IKr+/INCX+/If+/Ito+/IK1-/IKs-), 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.

Medical therapies for inflammatory bowel disease and liver dysfunction in an area with high prevalence of hepatitis B

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Background: Both the incidence and prevalence of inflammatory bowel disease (IBD) are rising globally, including Hong Kong. Considerable proportion of IBD patients requires immunosuppressive agents including steroids and biologics to control their disease activities. Reactivation of hepatitis B (HBV) on immunosuppressive agents has been reported in IBD patients but data from regions with high prevalence of hepatitis B are lacking.

Aim: To evaluate the effects of medical treatments on liver function in IBD patients and their potential association with chronic hepatitis B status in an area with high background prevalence of chronic hepatitis B.

Methods: All IBD patients attending the IBD Clinic in our hospital were included. We excluded patients with concurrent primary sclerosing cholangitis. Demographic data, viral hepatitis serological markers (HBsAg, anti-HBs, IgG anti-HBc and anti-HCV), liver function tests and their medical treatments (5-aminosalicylic acid [5-ASA], azathioprine, glucocorticoids, anti-TNF alpha) were evaluated.

Results: A total of 187 IBD patients were studied, of which 115 (61.5%) patients had ulcerative colitis (UC) and 72 (38.5%) patients had Crohn's disease (CD). There was a slight male predominance of 56.1% (n=105) and the mean age of these patients was 46 (±14) years. The median period of follow-up was 10 years (range, 0-48). Abnormal liver function tests (LFTs) with elevated alanine transaminase (ALT) were observed in 47 (25.1%) patients. Deranged LFT was more commonly seen in patients with CD (34.7%) than UC (19.1%; P=0.024). Thirty-five (74.5%), 16 (34%), 18 (38.3%), and 5 (10.6%) patients were taking 5-ASA, azathioprine, corticosteroids and anti-TNF agents, respectively. Eighteen (36.7%) patients were on single agent, 14 (28.6%) patients on two agents and 10 (20.4%) patients were on three or more agents. The hepatitis status of 145 (78.4%) patients were available and 8 (5.5%) of them were found to be HBsAg+. Thirty (20.7%) patients had prior exposure to hepatitis B virus (HBsAg- but anti-HBc+) and 5 (16.7%) of them had deranged LFT. No patients were positive against anti-HCV. In total, 4 (50%) HBsAg+ and 32 (23.4%) HBsAg- IBD patients had raised ALT (P=0.11).

Conclusions: Based on our data, abnormal LFTs were frequently found in IBD patients, particularly those with chronic hepatitis B. One-fifth of our IBD patients have serological evidence of prior HBV exposure. Close monitoring of LFTs and hepatitis B status are therefore advisable, particularly during treatment with immunosuppressants.

Use of aspirin in Chinese after recovery from primary intracranial haemorrhage

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Background and Objective: Intracranial haemorrhage (ICH) accounts for approximately 35% of all strokes in Chinese. Anti-platelet agent is often avoided after an index event due to the possibility of recurrent ICH.

Methods: This single-centred observational study included 440 consecutive Chinese patients with a first spontaneous ICH surviving the first month performed during 1996-2010. The subjects were identified, and their clinical characteristics, anti-platelet therapy after ICH, and outcomes including recurrent ICH, ischaemic stroke, and acute coronary syndrome were checked from hospital records.

Results: Of these 440 patients, 56 (12.7%) patients were prescribed aspirin (312 patient-aspirin years). After a follow-up of 62.2 ± 1.8 months, 47 patients had recurrent ICH (10.7%, 20.6 per 1000 patient years). Patients prescribed aspirin did not have a higher risk of recurrent ICH compared with those not prescribed aspirin (22.7 per 1000 patient-aspirin years vs 22.4 per 1000 patient years, P=0.70). Multivariate analysis identified age >60 years (HR=2.0; 95% CI, 1.07-3.85; P=0.03) and hypertension (HR=2.0; 95% CI, 1.06-3.75; P=0.03) as independent predictors for recurrent ICH. In a subgroup analysis including 127 patients with standard indications for aspirin of whom 56 were prescribed aspirin, the incidence of combined vascular events including recurrent ICH, ischaemic stroke, and acute coronary syndrome was statistically lower in patients prescribed aspirin than those not prescribed aspirin (52.4 per 1000 patient-aspirin years vs 112.8 per 1000 patient-years, P=0.04).

Conclusion: We observed in a cohort of Chinese post-ICH patients that aspirin use was not associated with an increased risk for a recurrent ICH.

Hyperthyroidism-induced left ventricular diastolic dysfunction: implication in hyperthyroidism-related heart failure

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Background: Heart failure occurs in 6% of hyperthyroid patients. Nonetheless only half of those with hyperthyroidism-related heart failure have impaired left ventricular (LV) systolic function. Thus diastolic dysfunction may play an important role in the pathogenesis.

Methods and Results: We performed serial echocardiographic examinations in 70 consecutive patients with hyperthyroidism (39 ± 2 years; 47 women) to determine their diastolic function and repeated the examinations 6 months after achieving a euthyroid state. All patients had normal LV systolic function, but diastolic dysfunction was detected in 22 cases (mild: 3, moderate: 15, and severe: 4). The prevalence of diastolic dysfunction increased with age from 17.9% in patients <40 years to 100% in those >60 years. Increasing age was the only independent predictor for diastolic dysfunction in hyperthyroid patients. After achievement of a euthyroid state, most patients (16/22, 72%) had completely normalised diastolic function: 100% of patients <40 years, 33.3% of those ≥60 years. Further analyses revealed significant age-related differences in the cardiovascular response to hyperthyroidism. Among patients <40 years, hyperthyroidism resulted in a marked reduction in total peripheral vascular resistance, increased cardiac output, and enhanced diastolic function as determined by E′. No such significant change in total peripheral vascular resistance or cardiac output was observed in hyperthyroid patients ≥40 years. In addition, hyperthyroidism was associated with reduced E′, signifying diastolic dysfunction in older hyperthyroid patients.

Conclusion: Hyperthyroidism is associated with diastolic dysfunction, particularly in older patients. It is partly reversible following achievement of a euthyroid state.

Potential roles of TRPV4 and TRPM2 channels in human embryonic stem cell-derived ventricular cardiomyocytes

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Vanilloid and malastatin transient receptor potential channels (TRPV and TRPM, respectively) are Ca^{2+}-permeable non-selective cation channels. These channels are regarded as environmental sensors in many types of cells. TRPV4 is thought to be involved in sensing osmolarity and membrane stretch. On the other hand, TRPM2 is known to be involved in sensing oxidative stress and reactive oxygen species (ROS), and is involved in apoptosis and necrosis. However, their roles in human embryonic stem cells (hESC) and hESC-derived ventricular cardiomyocytes (hESC-VCMs) are unknown. Our microarray data have shown the expression of TRPV4 and TRPM2 in hESC and hESC-VCMs. This was confirmed by RTPCR method in hESC. In functional studies of hESC-VCMs, 4α-phorbol 12,13-didecanoate (4α-PDD) [4 μM], a TRPV4 agonist elicited a rise of cytosolic Ca^{2+} that could be abolished by ruthenium red (RuR, 5 μM), a potent TRPV blocker. TRPV4 is known to be involved in sensing lower osmolarity. Interestingly, hypoosmolarity was found to induce a cytosolic Ca^{2+} rise in hESC-VCMs. These data suggest a role of TRPV4 in sensing osmolarity and cell swelling in hESC-CMs. For TRPM2-related functional studies, application of H2O2 (1 mM), a known TRPV4 activator, stimulated intracellular Ca^{2+} rises in hESC-VCMs. TM2E3, a TRPM2-specific blocking antibody designed to plug the ion permeation pore of TRPM2, inhibited H2O2-induced Ca^{2+} rises in hESC-VCMs, suggesting a functional role of TRPM2 in sensing oxidative stress in hESC-VCMs. Together these data show that endogenous TRPV4 and TRPM2 are important Ca^{2+} regulators in hESC-VCMs. While TRPV4 is involved in the osmo- and mechano-sensation, TRPM2 is involved in sensing oxidative stress in hESC-VCMs.

Development of proteomic surface barcodes in cardiomyocyte differentiation from human embryonic stem cells

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Introduction: Differentiation of human embryonic stem cells (hESCs) is an invaluable model for the investigation of molecular events during embryonic development, and supplies functional differentiated cells for regenerative therapies. Cardiomyocyte (CM) differentiation of hESC often yields heterogeneous cell populations, making it difficult to perform systematic, quantitative analyses of the differentiation process. There is also a need for improving differentiation and isolation procedures that generate highly pure populations of CM.

Methods: Surface proteins from hESC, and from differentiated CM were isolated by biotinylating proteins exposed on intact, viable cell population. The cells were lysed under stringent conditions, and the biotinylated proteins were purified by streptavidin affinity purification. The isolated proteins were then analysed by shotgun proteomics.

Results: This approach allows the characterisation of surface proteins that are differentially expressed in hESC and CM.

Conclusion: Combination of these differential expression patterns can be used as 'molecular barcodes' for the defining cell states and for purification of cells at different stages of differentiation.

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The association between glycated haemoglobin and waist circumference in the US population

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Introduction: Glycated haemoglobin (A1C) is now used for the diagnosis of diabetes and pre-diabetes. As these are related to obesity, we studied their relationship with waist circumference.

Methods: We analysed data on 960 men and 1001 women who participated in the United States National Health and Nutrition Examination Survey 2007-08. Participants who were older than 20 years, had overnight fasting, and had not been treated with anti-diabetic medication were included in the analysis.

Results: There was a continuous linear relationship between waist circumference and A1C, which was true both in men ($P=0.01$) and women (both $P<0.001$) after adjusting for age. The change in A1C due to a unit change in waist circumference was larger in men than in women. Moreover, age was an independent predictor of waist circumference in men ($P<0.001$) but not in women. The waist circumference corresponding to an A1C of 5.7% was 96.4, 101.7 and 107.0 cm in men aged 30, 50 and 70 years, compared to 95.1, 95.4, and 95.8 cm in women of the same ages respectively. The waist circumference corresponding to an A1C of 6.5% was 98.5, 103.8 and 109.1 cm in men aged 30, 50 and 70 years, compared to 101, 101.4, and 101.8 cm in women of the same ages respectively.

Conclusions: There is a linear relationship between A1C and waist circumference. A slimmer waist is associated with a lower A1C. Our results generally agree with the current waist circumference criteria of 102 cm and 88 cm for central obesity in American men and women, respectively. Young American men with a circumference even below 100 cm are already at risk from pre-diabetes and diabetes. In older American men, ageing alone confers a risk of diabetes, which means that they should also try to avoid abdominal obesity.

The pattern of non-intercepted medication errors in a university affiliated teaching hospital

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Introduction: The primary goal of reducing medication errors is to eliminate errors that reach the patient. We aimed to study the pattern of interception of medication errors along the medication use process.

Methods: We analysed reported medication incidents in a teaching hospital in 2006 to 2010. We used the 'Swiss Cheese Theory' to describe the interception of errors.

Results: Our analysis included 1268 in-patient and 303 out-patient errors. Among in-patient errors, 53.4% were prescribing, 29% were drug administration and 17.6% were dispensing errors. Of the in-patient errors, 26.8%, 4.9% and 2.4% related to drug administration, prescribing and dispensing, respectively, were not intercepted. Pharmacists intercepted 85.4% of the prescribing errors. Nurses detected 83% and 5% of the dispensing and prescribing errors respectively. Among out-patient errors, 91.4% were prescribing, 4.9% were drug administration, and 3.6% were dispensing errors. Of the out-patient errors, 4.6% and 3.0% related to drug administration and dispensing, respectively, reached the patients. Pharmacists intercepted 89.5% of the prescribing errors.

Conclusions: Having a preventive measure at each stage of the medication use process helps to prevent medication errors. However, many drug administration errors were not prevented and reached patients. Therefore, more interventions for preventing drug administration errors are warranted.

Medication incidents related to technology in a university-affiliated general hospital in 2006-2010

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Introduction: Technology often helps to reduce medication errors. The objective of this study was to assess medication errors in relation to technology used in the prescription or administration of medications.

Methods: Medication incidents reported during 2006–2010 in a university-affiliated general hospital were analysed. Computer-aided prescribing and medication label generation, 2-D bar-coded patient identification, parenteral drug administration devices were considered technology-related interventions.

Results: A total of 1538 medication incidents were reported; 17.3% of all incidents were technology-related, of which the majority were due to user errors (17%) rather than device errors (0.3%). 75.6% of the technology-related errors were prescribing errors, followed by drug administration (14.3%), dispensing (8.3%) and others (1.3%). 10.3% of all incidents were linked to computerised medication order entry, 4.2% to 2-D bar-coded patient identity, 1.3% to infusion pump devices and 1.2% to computer-aided medication label generation. The leading causes for technology-related errors included incorrect computer entry (49.3%), failure to comply with policies and procedures (39.4%), similar drug name (6.1%), device fault (1.5%), and lack of supervision (1.1%). 12% of the technology-related incidents were detected after the drug had been administered.

Conclusion: Technology may reduce medication errors but can also introduce new errors, which are mainly due to user mistakes. Therefore, when using technology-related interventions, careful and continuous monitoring is still needed in order to eliminate medication errors.

65 Reducing the use of inappropriate abbreviations in prescriptions

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Introduction: Inappropriate use of abbreviations in prescriptions affects patient safety.

Objectives: We investigated the effect of the 'Do Not Use' list on the use of such inappropriate abbreviations and the adherence to the Hospital Authority's approved 'Standard Abbreviations in Prescribing' list.

Methods: We analysed the use of prescribing abbreviations in prescriptions before and after the introduction of the 'Do Not Use' list and the rate of related medication incidents in Queen Mary Hospital over this period.

Results: A total of 12 639 drug items were analysed. The use of abbreviations discouraged by the 'Do Not Use' list was 9% per number of drugs prescribed before its introduction in October 2008, which was reduced to 1% after the intervention. Commonly used abbreviations related to the 'Do Not Use' list were 'QD', 'mcg' and 'units', which were used at a rate of 5.5%, 1% and 2% at baseline and reduced to 0.5%, 0.3% and 0.4% respectively after its introduction. 8% of the drugs were abbreviated drug names of which 49% were not found in the 'Standard Abbreviations' list. Non-approved abbreviations were also used to indicate the 'route of administration' at a rate of 10%. During 2008–2010, one medication incident directly related to the 'Do Not Use' list and 16 incidents related to the use of other non-approved abbreviations (four incidents having a severity score of two or more and 13 incidents scoring one) were reported.

Conclusions: The introduction of the 'Do Not Use' list was very effective in reducing the use of inappropriate abbreviations. However, the use of non-approved abbreviations is common. Enlarging the 'Do Not Use' list is recommended.

66 Evidence of serologic activity in chronic hepatitis B after hepatitis B surface antigen seroclearance

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Background: Possible serologic activity after hepatitis B surface antigen (HBsAg) seroclearance in chronic hepatitis B (CHB) has not been thoroughly investigated.

Methods: We determined the levels of serum HBV DNA, hepatitis B core-related antigen (HBcrAg) and linearized HBsAg (CLEIA prototype) in CHB patients after HBsAg seroclearance. A total of 329 CHB patients (72.0% male) with documented HBsAg seroclearance were recruited.

Results: The median time interval from presentation to HBsAg seroclearance was 69.4 months. The median age of HBsAg seroclearance was 50 years. Serum HBV DNA, HBcrAg and linearized HBsAg were performed at a median time interval of 11.2 months after HBsAg loss. 85 (25.8%) and 69 (21%) of patients had detectable linearized HBsAg and HBcrAg, respectively. 133 patients (40.4%) had either one or both serologic markers detectable whereas serum HBV DNA was detectable in only seven (2.1%) patients. There was no correlation between linearized HBsAg and HBcrAg levels (r=0.095, P=0.924). There was no difference in the incidences of detectable linearized HBsAg and HBcrAg between patient samples taken at 6-12 and >12 months after HBsAg seroclearance (P=0.146 and 0.079 respectively). Among patients with detectable serology, an increased time interval after HBsAg seroclearance was not associated with any significant change in median levels of linearized HBsAg (P=0.581) and HBcrAg (P=0.951).

Conclusion: Using these novel linearized HBsAg and HBcrAg assays, viral serologic activities were demonstrated in more than 40% of CHB patients after HBsAg seroclearance. These tests may have potential applications in diagnosing and prognosticating CHB patients with HBsAg seroclearance.

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A large case-control study on the predictability of hepatitis B surface antigne levels 3 years before HBsAg seroclearance

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Background:. The kinetics of hepatitis B surface antigen (HBsAg) levels prior to spontaneous HBsAg seroclearance has not been fully investigated.

Methods: The kinetics of HBsAg and HBV DNA of 203 treatment-naïve, hepatitis B e antigen (HBeAg)–negative patients with spontaneous HBsAg seroclearance were compared with 203 age-, sex-matched HBeAg-negative controls. Serum samples at 3 years, 2 years, 1 year and 6 months before HBsAg seroclearance and at the time of HBsAg loss were tested.

Results: The median HBsAg levels at these respective time points before HBsAg seroclearance were 23.5, 3.51, 0.524 and 0.146 IU/mL. For all time points, patients with HBsAg seroclearance had significantly lower median HBsAg and HBV DNA levels compared to those of the controls (all P<0.001). The median HBsAg and HBV DNA levels declined significantly until HBsAg seroclearance (P<0.001). Although median HBsAg levels also decreased significantly with time (P=0.006) in controls, median HBV DNA levels remained similar (P=0.414). Significant correlation between HBsAg and HBV DNA levels were found in both groups, but the correlation was much better in patients with HBsAg seroclearance (r=0.605) compared to controls (r=0.294). Serum HBsAg levels, followed by HBsAg log reduction, were the best predictors of HBsAg seroclearance (AUROC 0.833 and 0.803 respectively). The optimal cutoff HBsAg level to predict HBsAg seroclearance was <200 IU/mL (sensitivity 84.2%, specificity 73.4%). For patients with HBsAg level ≥200 IU/mL, an annual 0.5 log reduction was predictive of subsequent HBsAg seroclearance (AUROC 0.867).

Conclusion: Serum HBsAg <200 IU/mL and 0.5 log reduction in HBsAg were predictive of HBsAg seroclearance within 3 years of follow-up. These parameters may serve as good indicators for the consideration of treatment duration and cessation for chronic hepatitis B.

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Hepatitis B surface antigen levels predict minimal histologic changes in hepatitis B e antigen positive chronic hepatitis B

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Background: The association between hepatitis B surface antigen (HBsAg) levels and histologic abnormalities in hepatitis B e antigen (HBeAg)-positive chronic hepatitis B (CHB) has not been determined.

Methods: Serum HBsAg and HBV DNA levels were determined in 140 HBeAg-positive CHB patients (65% male) with liver biopsy performed. The upper limit of normal (ULN) of serum alanine aminotransferase (ALT) was set at 30 and 19 U/L for men and women respectively. Serum HBV DNA levels were performed using Cobas Taqman assay (lower limit of detection 20 IU/mL). HBsAg titers were assayed using Roche Elecsys HBsAg II assay (lower limit of detection, 0.05 IU/mL).

Results: The median age of patients was 32.7 years. Seventeen (12.1%) and 39 (27.9%) patients had normal ALT and ALT 1-2xULN respectively. Sixty-eight (48.6%) and 72 (51.4%) had minimal necroinflammation (Knodell Histology Activity Index <7) and fibrosis (Ishak Fibrosis Score <2) respectively. Patients with ALT≤2xULN had significantly higher median HBsAg levels when compared to patients with ALT >2xULN (52 535 and 9362 IU/mL respectively, P<0.001). Among patients with ALT ≤2xULN, serum HBsAg levels achieved an area under receiver operating characteristic curve of 0.808 and 0.869 in predicting minimal necroinflammation and fibrosis respectively. Using the cutoff HBsAg level of 50 000 IU/mL in patients with ALT≤2xULN, positive and negative predictive values for predicting minimal necroinflammation and fibrosis were 89.5% / 63.0% and 93.8% / 61.1% respectively.

Conclusion: Among HBeAg-positive patients with ALT≤2xULN, serum HBsAg levels can accurately predict minimal changes in histology, and could play a role in treatment commencement decisions.

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Serum hepatitis B surface antigen kinetics in hepatitis B e antigen–negative chronic hepatitis B

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Background: The differences in serum hepatitis B surface antigen (HBsAg) kinetics among different levels of viremia in hepatitis B e antigen (HBeAg)-negative CHB have not been thoroughly investigated.

Methods: We recruited 100 HBsAg-positive, HBeAg-negative treatment-naïve CHB patients noted with undetectable HBV DNA (≤20 IU/mL) at three consecutive time points (Group A). The results were compared with two age- and sex-matched groups: baseline HBV DNA 20-2000 IU/mL (Group B, n=100) and baseline HBV DNA >2000 IU/mL (Group C, n=100). The median duration of follow-up was 21.4 (range, 10.8-51.9) months.

Results: The median HBsAg levels in Group A were significantly lower than Groups B and C at all time points (P<0.001). Group B patients had the highest median HBsAg / HBV DNA ratio (P<0.001). There was a significant decrease in HBsAg levels and HBsAg / HBV DNA ratio in Group A (P<0.001), while both markers in Groups B and C remained similar (P>0.05). When compared with other patient subgroups, the combination of persistently undetectable HBV DNA with HBsAg <100 IU/mL was associated with increased HBsAg reduction (0.341 log IU/mL/year, P=0.002). HBsAg reduction was further increased among HBsAg 10-<100 IU/mL (0.683 log IU/mL/year). Among patients with persistently undetectable HBV DNA and HBsAg <100 IU/mL, baseline HBsAg levels achieved an area under curve of 0.876 in predicting >1 log annual HBsAg reduction, with HBsAg 10-<100 IU/mL being the optimal level for prediction (sensitivity 90%, specificity 74.6%).

Conclusion: The combination of persistently undetectable viremia and HBsAg <100 IU/mL in HBeAg-negative CHB was predictive of subsequent increased HBsAg reduction. Serial monitoring of serum HBsAg in selected patient groups could have important prognostic significance and potential in guiding nucleoside analogue therapy in CHB.

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Continuous entecavir for treatment-naïve chronic hepatitis B: the 4-year results

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Background: Long-term data of uninterrupted entecavir for treatment-naïve chronic hepatitis B (CHB) patients up to 4 years are lacking.

Objectives: To determine the serologic, biochemical, virologic responses, resistance profile and safety of continuous entecavir up to 4 years.

Methods: A total of 222 CHB patients were treated continuously with entecavir 0.5 mg daily for up to 4 years. The cumulative rates of HBeAg seroconversion, ALT normalisation, DNA undetectability and entecavir signature mutations up to year 4 were determined. HBV DNA levels were measured by Roche Taqman real time PCR assay (lower limit of detection, 12 IU/mL). Resistance profile was determined by line probe assay (LiPA) for patients with detectable HBV DNA.

Results: A total of 157 male and 65 female patients, with a median age of 45 years, were recruited. The median duration of follow-up was 36.2 months. 222, 188, 172 and 70 patients were followed up for 1, 2, 3 and 4 years respectively. HBV DNA became undetectable in 81.1%, 90.4%, 93.0% and 95.7% from years 1 to 4. The ALT normalisation rate for 181 patients with elevated baseline ALT were 84.0%, 88.8%, 91.2% and 91.2% from years 1 to 4. 90 (40.5%) patients were HBeAg positive at baseline, with HBeAg seroconversion rate of 22.2%, 40.8%, 52.9% and 51.7% from years 1 to 4. One patient developed HBsAg seroconversion at year 2. Virologic breakthrough (>1 log HBV DNA increase from the nadir) was noticed in four patients; one patient developed entecavir signature mutation at year 3, resulting in a cumulative resistance of 0.6% up to year 4. There were no serious adverse events related to the drug.

Conclusion: Even when using very sensitive assays for HBV DNA and viral resistance measurements, continuous entecavir up to 4 years achieved a more than 95% chance of undetectable HBV DNA and only a 0.6% probability of resistance.

Acknowledgements: The assays used to determine HBV DNA levels and viral resistance in our laboratory are supported by an unrestricted grant from Bristol-Myers Squibb Company.

Cerebrospinal fluid biomarkers in Chinese Alzheimer's disease patients

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Introduction: Cerebrospinal fluid (CSF) beta-amyloid (Aβ42), total tau (tau) and phosphorylated-tau 181 (p-tau 181) were reported to be pathological biomarkers useful in the diagnosis of Alzheimer's disease (AD) in Caucasian populations. There are limited data in Asian populations, and the validity of these biomarkers has not been established in Chinese AD patients. The objective of the present study was to investigate the validity of using CSF Aβ42, tau and p-tau 181 as biomarkers in the diagnosis of AD in Chinese patients.

Methods: In this cross-sectional study, we recruited both AD patients and non-AD subjects. Alzheimer's disease subjects were diagnosed in accordance to the NINCDS-ADRDA criteria, and non-AD control subjects did not fulfil these AD criteria. The research protocol was approved by the HKU/HA HKW IRB, and written informed consents were obtained from all subjects. Cerebrospinal fluid samples were obtained by lumbar punctures, and were stored (in aliquots) are stored at -80°C until assay. The three CSF biomarkers (Aβ42, tau and p-tau 181) were assayed by ELISA, using commercial kits (INNOTEST hTau Ag, INNOTEST PHOSPHO-TAU (181P), INNOTEST ß- AMYLOID1-42; supplied by Innogenetics NV, Belgium). The levels of the CSF biomarkers were compared between the AD and non-AD, using Mann-Whitney *U* test. Multivariate logistic regression of each biomarker (adjusted for age and gender) was then performed.

Results: Among 34 subjects recruited, 19 had AD and 15 had non-AD (5 cognitively normal and 10 non-AD dementia). The mean age of AD and non-AD subjects were 81.3 ± 8.3 and 76.3 ± 7.8 years (P=0.088), respectively. The intra-assay coefficient of variation (CV) were 3.2%, 1.4% and 7.6% for tau, p-tau 181 and Aβ42, respectively. In bivariate analyses, there were no significant differences in the tau and p-tau levels between the two groups. For Aβ42, a non-significant trend was present (P=0.06), with a lower level in the AD versus non-AD groups (354 ± 197 pg/mL versus 463 ± 152 pg/mL). There were significant differences in both the CSF Aβ42/tau ratio (3.6 ± 2.7 vs 6.4 ± 4.6; P=0.03) and Aβ42/p-tau ratio (8.4 ± 4.1 and 13.8 ± 7.1; P=0.01) between AD patients and non-AD patients. After adjustment for age and gender, the Aβ42/p-tau ratio was an independent predictor of AD (P=0.02).

Conclusion: We conclude that the Aβ42/p-tau ratio is a potentially useful diagnostic biomarker of AD in our Chinese population.

Acknowledgement: Grant from HKU AD Research Network, SRT Healthy Aging, HKU.

Fractional 1435-nm diode laser system for skin rejuvenation in Chinese patients

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Background: Fractional 1435-nm diode laser system is commercially available. The objective of the study was to assess the efficacy and adverse effects of a 1435-nm laser system for skin rejuvenation in Chinese patients.

Methods: A fractional diode laser with a wavelength of 1435 nm and a maximum pulse energy of 9 mJ was used for skin rejuvenation. Ten subjects were recruited. Four treatment sessions over an interval an interval of 14 days were performed. The physician selected a use-selectable setting of low (4 mJ) / medium (7 mJ) / high (9 mJ) intensity on the hand piece and eight passes were delivered. Standardised photographs were taken at baseline, 2 weeks post-treatment and 1 month after the last treatment. Pain related to the treatment was assessed by the patient using a visual analogue scale (0-10), redness, swelling and heat sensation with a severity scale of 0-3. Two independent clinicians assessed the photographs for efficacy and adverse effects.

Results: Skin texture improved after two treatments with P values of 0.007, 0.0114, 0.006 at each follow-up, respectively. Improvement in pigmentation was also significant after two treatments with P values of 0.007, 0.020, 0.010. Improvement in wrinkles was observed at 1 month after the last treatment (P=0.046). Mild erythema was the most common adverse effect in 50% 1 month post 4th treatment.

Conclusions: Fractional 1435-nm laser system is effective for the improvement of skin texture, pigmentation and wrinkles in Chinese patients after a course of four treatments.

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Safety and efficacy of a minimally invasive radiofrequency device for skin tightening in Chinese patients

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Background: This radiofrequency device stimulates the regeneration of new collagen, elastin and hyaluronic acid for the treatment of ageing skin. This study assessed the safety and efficacy when used for the treatment of skin tightening in Chinese patients.

Methods: Fifteen recruited subjects (mean age, 53 years) were offered one treatment. The cartridge consists of five pairs of 32°, bi-polar radiofrequency needles 1-2 mm which was inserted into the skin. Radiofrequency energy was delivered for a specific period of time at predetermined temperature settings. Patients' subjective assessment was recorded using a questionnaire. Standardised clinical photographs were taken at baseline, immediately after treatment, 1 day, 3 days, 1 week, 1 month, 3 months and 6 months post-treatment. These photographs were assessed by two independent physicians.

Results: Subjects reported pain, swelling, redness and bruising which slowly resolved over time. There was one case of atrophic scar noticed at 3-month and 6-month follow-up. The level of satisfaction was 73% at 1-month follow-up and increased to 89% at 6-month follow-up. Physician assessment showed similar findings in terms of adverse effects. Both physicians observed statistically significant ($P<0.05$) skin tightening when assessing the jaw line, cheeks, nasolabial fold as well as oral commissures at 1-, 3- and 6-month follow-up. Improvement in fine lines in the lower face was also noticed 3 and 6 months post-treatment (both $P=0.024$), whilst improvement in skin texture was seen at 6-month follow-up ($P=0.046$).

Conclusion: Minimally invasive radiofrequency device for skin tightening is effective in Chinese patients. The common adverse effects are pain, edema, erythema and bruising.

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The efficacy of a dual filter handpiece (500-670 nm and 870-1200 nm) of an intense pulse light device for the treatment of facial telangiectasia

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Background: The dual filter handpiece with wavelength 500-670 nm and 870-1200 nm, pulse width 5-100 ms and spot size 10x15 mm can deliver energy up to 80 J/cm^2. The objective of the study was to assess the safety and efficacy of this handpiece for the treatment of facial telangiectasia.

Methods: Ten subjects received up to four treatments with an interval of 4-6 weeks. Only areas with facial telangiectasia were treated and the parameter used was 34-40 mJ/cm^2, 10 ms. At each visit, patient questionnaires were given for objective assessments and standardised clinical photographs were taken for two independent physicians' review.

Results: Mild pain and erythema are common adverse effects that resolve spontaneously. At 1 week post-treatment, one out of 18 treatment sessions developed crusting at the nasal region and seven out of 18 treatment sessions developed crusting at the cheeks. Hypopigmentation was noticed in one out of 18 treatment sessions and hyperpigmentation was noticed in three out of 18 treatment sessions at the cheeks. In terms of efficacy, statistically significant improvement was found after the second and third treatments.

Conclusion: Dual filter handpiece with wavelength 500-670 nm and 870-1200 nm seems to be effective for the treatment of facial telangiectasia. Mild crusting was observed which may be overcome by adjusting the parameters used.

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The efficacy of a 915-nm laser, 650-nm LED light source with mechanical massage device for the treatment of cellulite and circumferential reduction in Chinese patients

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Objective: To assess the efficacy of a 915-nm laser, 650-nm LED light source with mechanical massage device for the treatment of cellulite and circumferential reduction of the thigh after a course of eight treatments.

Methods: Ten subjects with cellulite stage 1 or above based on the Nurnberger-Muller Scale were recruited for the study. A series of eight treatment sessions were offered to one thigh of the subjects with two sessions per week at least 1 day apart. The non-treated thigh served as the control. Standardised clinical photos, the stage of the cellulite and circumference of the thigh were recorded at each visit as well as 1 and 3 months after the last treatment. At each visit, the subjects filled out a patient questionnaire and two independent physicians assessed the standardised photographs.

Results: There was no improvement in cellulite grading according to the physicians' assessment of the clinical photos. Subjects rated the results better with two reporting a 1-24% improvement and one 25-49% improvement after four treatments. Only one subject completed all eight sessions and rated her improvement the best, 50-74%. Two cases of mild bruising were observed: one after the first treatment session, the other after the third treatment session. Both subsided spontaneously.

Conclusion: The result demonstrates that patient satisfaction was achieved. Mild bruising can develop following the procedure.

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Evaluation of platelet inhibition with point-of-care device VerifyNow in local Chinese patients with acute coronary syndrome treated with clopidogrel and prasugrel: a single centre cohort study

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Introduction: Clopidogrel has been used widely in the treatment of acute coronary syndrome but there is increasing evidence concerning its limited antiplatelet activity especially in Asian population. Prasugrel has been shown to have superior efficacy in platelet inhibition compared to clopidogrel and it has been translated to improved clinical outcomes but increased bleeding risk.

Methods: From December 2010, Chinese patients admitted with acute coronary syndrome who are clopidogrel or prasugrel naïve were treated with either loading dose of clopidogrel 300 mg or 600 mg, or prasugrel 60 mg at physicians' discretion. Antiplatelet effects were evaluated by VerifyNow P2Y12 at 4 to 6 hours and 24 hours post-loading.

Results: A total of 49 patients were recruited into study. Fourteen patients received clopidogrel 300 mg, 24 patients received clopidogrel 600 mg, and 11 patients received prasugrel 60 mg. Mean platelet reactivity units (PRU) at 4 to 6 hours and 24 hours in clopidogrel 300 mg group are 291 ± 79 and 237 ± 103, respectively, in clopidrgrel 600 mg group 308 ± 95 and 286 ± 99 respectively, in prasugel 60 mg group 51 ± 96 and 17 ± 21 respectively. Four to 6 hours and 24 hours PRU in prasugrel group are significantly lower than that in clopidogrel 300 mg and 600 mg group ($P<0.005$). The percentage of clopidogrel hyporesponsiveness (as defined by PRU >240) are 73.7% at 4-6 hours and 52.6% at 24 hours while percentage of prasugrel hyporesponiveness is 9.1% at 4-6 and 0% at 24 hours.

Conclusions: A loading dose of prasugrel 60 mg in local Chinese patients with acute coronary syndrome has significantly more potent and rapid antiplatelet effect than loading clopidogrel 300 mg or 600 mg. The proportion of clopidogrel hyporesponsiveness in local Chinese patients is high.

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Effect of somatosensory stimulation on upper limb motor function in stroke survivors: a pilot study

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Introduction: Upper limb motor recovery is usually unsatisfactory after stroke. This study tested whether a single 2-hour session of peripheral nerve stimulation (PNS) by portable neurostimulator normally used for transcutaneous nerve stimulation (TENS) could improve pinch strength and function of the upper limb.

Methods: Twelve patients with subacute stroke were randomly assigned into two groups (n=6 in each group). Each received a single 2-hour session of PNS to the median and ulnar nerve of the affected upper limb. Group A received PNS by portable TENS device (TENS-PNS). Group B received sham stimulation (control). Pinch strength, grip strength and Jebsen-Taylor Hand Function Test (JTHFT) were measured before and after the simulation.

Results: Pinch strength increased after stimulation in the TENS-PNS group (median, 0.15 kg; interquartile range, 0.15-0.80 kg) while that for control group decreased. Post-hoc Mann-Whitney-Wilcoxon test showed the difference in change of pinch strength was statistically significant when comparing TENS-PNS with control group (P=0.036). There were no statistically significant differences in grip strength and JTHFT.

Conclusion: Peripheral nerve stimulation using portable neurostimulator could be considered as a modality for improving upper limb impairment in patients with stroke. Further studies using larger sample size is required to further confirm the effect of this modality.

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Probing the mechanobiological properties of human embryonic stem cells in cardiac differentiation by optical tweezers

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Human embryonic stem cells (hESC) and hESC-derived cardiomyocytes (hESC-CM) hold great promise for the treatment of cardiovascular diseases. However, the mechanobiological properties of hESC and hESC-CM remain elusive. In this paper, we examined the dynamic and static micromechanical properties of hESC and hESC-CM, by manipulating via optical tweezers at the single-cell level. Theoretical approaches were first developed to model the dynamic and static mechanical responses of cells during optical stretching. Our experiments showed that the mechanical stiffness of hESC increased after cardiac differentiation. Such stiffening could associate with myofibrillar assembly that underlies the functional characteristics of contracting hESC-CM. In summary, our findings lay the ground work for using hESC-CMs as models to study mechanical and contractile defects in heart diseases.

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Suppression of hyaluronan using 4-methylumbelliferone is associated with reduced renal inflammation and fibrosis in NZBWF1/J mice

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Introduction: Accumulation of hyaluronan (HA) in tissues is associated with inflammation. We have previously demonstrated that intra-glomerular expression of HA is increased in patients with lupus nephritis during active disease, but its role during pathogenesis of disease remains to be elucidated. 4-methylumbelliferone (MU) is a specific inhibitor of HA synthesis. In this study, we determined the effect of MU on clinical, serological and histological parameters in NZBWF1/J mice, with particular emphasis on inflammatory and fibrotic processes.

Methods: Female NZBWF1/J mice with established disease (proteinuria >3 g/L) were randomised into three groups. Mice were treated daily with PBS, 1% Arabic Gum in PBS (Gum) or MU in Gum (3 g/kg/day) for 2, 4, 8 and 12 weeks.

Results: In MU-treated mice, serum HA levels and intra-glomerular expression of HA were reduced after 12 weeks' treatment, which was associated with decreased anti-dsDNA antibody production and improved renal function and histology compared to PBS- or Gum-treated mice. Treatment of MU also reduced intra-glomerular infiltration of B cells, T cells and macrophages. In addition, MU-treated mice showed reduced gene and protein expression of cytokines and matrix proteins such as MCP-1 and collagen type III compared to PBS- and Gum-treated mice.

Conclusion: Our findings demonstrate that HA plays an important role in inflammation and fibrosis in lupus nephritis and may be a potential therapeutic target in patients with lupus nephritis although further studies are warranted to confirm this.

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Distinct immunomodulatory effect of human embryonic stem cells (hESC) and hESC-derived cardiomyocytes on human dendritic cells and natural killer cells

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Introduction: Transplantation of human embryonic stem cells (hESC)–derived ventricular cardiomyocytes (vCM) to damaged heart provides a therapeutic strategy for cardiac tissue regeneration. However, immunologic acceptance of the transplanted vCM remains a challenging aspect. Dendritic cells (DC) are the major antigen presenting cells to T cells and are key in triggering both the innate and adaptive immune responses. In addition to killing tumour and virus-infected cells, natural killer (NK) cells distinguish allogeneic major histocompatibility complex (MHC) and participate in transplant rejection. Here we examined the immunomodulatory properties of hESC and hESC-vCM in modulating human DC and NK cells functions.

Methods: hESC (HES2) were cultured and differentiated into vCM using protocols established in the Li Lab as extensively described. Human immature DC were generated by positively selected CD14 cells from peripheral blood mononuclear cells (PBMC) in culture with IL-4 and GM-CSF. Human NK cells were isolated from PBMC. hESC or hESC-vCM were co-cultured with DC in the presence or absence of lipopolysaccharides (LPS). DC maturation was measured as surface expression of DC activation markers, ie CD80, CD86 and MHC class II. Cytokines level of IL-6, IL-10, TNF-alpha and TGF-beta in the co-culture supernatants was also measured. NK cytotoxicity towards hESC and hESC-vCM were performed.

Results: hESC inhibited LPS-induced DC maturation. DC co-cultured with hESC exhibited lower surface expression of CD86 and MHC class II with a decreased production of TNF-alpha and increased level of TGF-beta. hESC underwent minimal killing by NK cells. By contrast, hESC-vCM activated DC to undergo maturation and were susceptible to killing by NK cells.

Conclusion: hESC and hESC-vCM posses distinct immunomodulatory property in modulating DC and NK cells functions. Our data lay the groundwork for immune cells-specific modulation for prolonging hESC-vCM graft survival and efficacy.

Engineering micro-alignments of 2- and 3-D hESC-derived ventricular tissues to reproduce anisotropic properties of the native heart: an accurate arrhythmias model for cardiotoxicity screening

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Introduction: In the native heart, ventricular CMs are aligned in a highly organised structured manner such that the conduction of electrical signals is anisotropic for directional and coordinated contractions to effectively pump blood. In other words, electrical conduction is asymmetrical (ie anisotropy) with distinct transverse and longitudinal velocities. Unlike the native ventricle, clusters of hESC-CMs differentiated using either the EB formation or directed differentiation are random structures with NO obvious organisation and anisotropy as we previously published.

Methods: Using a microgroove technology, here we engineered organised 2- and 3-D hESC-derived ventricular strips, followed by high-resolution optical mapping recordings to examine in details their action potential and conduction properties.

Results: Compared with randomly distributed unaligned controls, hESC-CMs seeded on micro-fabricated polydimethylsiloxane (PDMF) consisting of nanometer-microgrooves induced cell alignment with more organised sarcomeric structures. Functionally, hESC-CMs seeded on PDMS with 5 minutes plasma treatment has shown prominent anisotropic propagation with faster longitudinal conduction velocity (LCV, 5.9 ± 0.60 cm/sec) than transverse conduction velocity (TCV, 3.2 ± 0.48 cm/sec), resulting a anisotropic ratio (AR) of 1.84, which is similar with the natural AR (~2) in ventricles.[1,2] Using programmed electrical stimulations (ie the standard S1, S2, S3 stimulating protocol), the inducibility of 2-D hESC-CMs monolayer to generate reentrant arrhythmia was also investigated. Among the aligned 2-D models, spiral wave was only induced on one out of five (20%) monolayers, while four out of seven (57%) for unaligned controls. Combined with our preliminary data on neonatal rat ventricular monolayer (NRVM) that 25% of aligned NRVMs can be induced of reentrant spiral waves versus a 75% of control NRVMs, we conclude that cells with successfully induced alignment are less vulnerable of arrhythmias than unaligned controls. Together with the technology of biomedical engineering, we have also successfully developed a reproducible method to build 3-D cardiac strip consisting of hESC-CMs, which showed a conduction velocity of 7.3 cm/sec (n=1) under physiologic (37 degree) condition.

Conclusion: Our micro-nanofabrication strategy has successfully functionally reproduced anisotropic properties. These results will be crucial for future development of viable cardiac patches for myocardial repair with improved efficacy and safety, and for rendering cardiotoxicity screening, prediction assays and heart disease model more accurate and indicative of the native heart.

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Occult hepatitis B infection and HBV replicative activity in patients with cryptogenic cause of hepatocellular carcinoma

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Objective: We aimed to investigate the incidence of occult hepatitis B infection (OBI) in patients with 'cryptogenic' hepatocellular carcinoma (HCC) and to study the HBV replicative activity in these patients.

Methods: Tumourous and adjacent non-tumourous liver tissues were obtained from 33 cryptogenic HCC patients and 28 HCC patients with identifiable causes (13 with chronic hepatitis B [CHB], 6 with chronic hepatitis C, and 9 alcohol-related).

Results: Occult hepatitis B infection was identified by nested PCR. Intrahepatic HBV DNA, covalently closed circular DNA (cccDNA), and pregenomic RNA (pgRNA) were quantified by real-time PCR and RT-PCR respectively. Occult hepatitis B infection was identified in 24 (73%) cryptogenic HCC patients, one (17%) HCC patient with HCV and five (56%) patients with alcohol-related HCC. Cryptogenic HCC patients with OBI had lower intrahepatic total HBV DNA levels than HCC patients with CHB (median, 0.010 vs 3.19 copies/cell, respectively; P<0.0001). Only six (26%) cryptogenic HCC patients with OBI had detectable cccDNA (median, <0.0002 copies/cell), which was significantly lower than that of the CHB patients (median, 0.005 copies/cell; P<0.0001). HBV pgRNA were detectable in 12 (52%) cryptogenic HCC patients with OBI (median, 0.0001 copies/cell), which was significantly lower than that of the CHB patients (median, 3.24 copies/cell; P<0.001).

Conclusion: 73% of patients with apparently unidentifiable causes for HCC were HBV-related. The low intrahepatic HBV DNA and pgRNA levels indicated that persistent viral replication and possibly HBV integration are the likely causes of HCC in OBI patients.

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Effect of nucleos(t)ide analogues therapy on HBsAg, intrahepatic HBV DNA and covalently closed circular DNA levels

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Objective: We aimed to study (1) the effects of 1-year nucleos(t)ide analogue (NA) therapy on HBsAg and covalently closed circular DNA (cccDNA) levels; and (2) the possible use of HBsAg reduction as a marker for cccDNA reduction.

Methods: We recruited 124 NA-treated patients with baseline and 1-year sera and liver biopsies. The NAs were categorised into the more potent (entecavir, telbivudine, and clevudine; n=71) and less potent groups (lamivudine and adefovir; n=53). cccDNA and HBsAg levels were measured by real-time PCR and the Elecsys HBsAg II assay, respectively.

Results: At year 1, there were approximately 5 $\log_{10}$IU/mL, 2 $\log_{10}$copies/cell, and 1 $\log_{10}$copies/cell reductions in serum HBV DNA, intrahepatic total HBV DNA, and cccDNA, respectively. Only a small reduction of HBsAg (mean, 0.18 $\log_{10}$IU/mL) was observed. There were no significant differences between the more and less potent NAs in the reduction of HBsAg, intrahepatic total HBV DNA and cccDNA. Although 88/124 (71%) patients had undetectable serum HBV DNA, all had detectable HBsAg and intrahepatic total HBV DNA. Logarithmic reductions of HBsAg and cccDNA correlated weakly (r=0.183, P=0.042). Patients with cccDNA reduction ≥0.87 $\log_{10}$copies/cell (the median level) had a greater HBsAg reduction than patients with cccDNA reduction <0.87 $\log_{10}$copies/cell (0.383 vs -0.015 $\log_{10}$IU/ml, respectively; P=0.003).

Conclusion: Despite the profound serum HBV DNA reduction after 1 year of therapy, reduction in HBsAg level was minimal, and reduction in intrahepatic total HBV DNA and cccDNA was relatively mild. HBsAg reduction may be a potential marker for the monitoring of cccDNA reduction during NA therapy.

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Age does not affect efficacy and survival outcomes of advanced hepatocellular carcinoma patients on sorafenib

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Introduction: With the ageing population, hepatocellular carcinoma (HCC) in the elderly population represents a significant health burden. However, elderly patients are under-represented in clinical trials. It is not clear whether age affects the outcome of advanced HCC patients on sorafenib.

Methods: Data from a prospectively maintained database containing a consecutive cohort of advanced HCC patients, with Child-Pugh A or B liver function and Eastern Cooperative Oncology Group (ECOG) performance status of 0-2 treated with sorafenib, were analysed. The patients were categorised into older (age ≥70) and younger (age <70) groups. Univariate and multivariate regression models were used to study the effect of age.

Results: A total of 172 patients, respectively 35 in the elderly (median age, 73) and 137 in the younger group (median age, 55) were analysed. Disease control rate was similar in the older and younger groups (24.8% vs 14.3%, P=0.258). The median progression-free survival was also comparable (2.99 vs 3.09 months, P=0.275), as was the overall survival (5.32 vs 5.16 months, P=0.310). Age group did not have a significant effect on both OS and PFS after adjusting for other risk factors (P=0.369 and 0.552 in OS and PFS respectively).

Conclusions: The efficacy and survival outcomes were similar in elderly and young advanced HCC patients treated with sorafenib.

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Triple-negative breast cancer may arise de novo as reflected by low frequency of associated in-situ component

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Introduction: In general, breast cancer carcinogenesis involves a multi-step sequence, initiating from premalignant stages, progressing on to preinvasive ductal-carcinoma-in-situ (DCIS), then to invasive ductal carcinoma (IDC) associated with DCIS (IDC/DCIS). Triple-negative breast cancer is a distinct entity characterised by its aggressive behaviour. The frequency of associated DCIS with its implications in carcinogenesis in triple-negative breast cancer is not known.

Methods: Tumour data obtained from 1355 consecutive female patients undergoing upfront surgery for primary breast cancer were analysed; 196 patients with pure DCIS were excluded. Tumour samples were divided into three groups based on differential immunohistochemical expression patterns: (i) hormone-dependent (ER and/or PR-positive, and HER2-negative), (ii) HER2-positive, and (iii) triple-negative. Frequency of associated DCIS, other clinical and biological parameters were compared.

Results: The number of patients with hormone-dependent, HER2-positive and triple-negative subgroups were 661, 258 and 117 respectively. Less than a third of all triple-negative tumours had associated DCIS. Triple-negative tumours had higher Ki67 levels than other subgroups (Spearman correlation coefficient 0.46, P<0.01), more likely of higher histological grade (Spearman correlation 0.25, P<0.01) and less frequently associated with DCIS (Spearman correlation -0.13, P<0.01).

Conclusions: It may be postulated that triple-negative breast tumours arise de novo due to a drastic genetic event rather than via the DCIS- IDC/DCIS sequence. This is in concordance with current evidence that major defects in tumour suppressor genes such as *TP53* and *BRCA* are observed in these tumours.

A phase I/II study of foretinib, a multikinase inhibitor of MET, Tie-2 and VEGFR in advanced hepatocellular carcinoma (Hcc)

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Background and Objectives: Foretinib is an oral multikinase inhibitor targeting c-Met, Tie-2, RON, Axl, and VEGFR. HGF/Met signalling plays a pivotal role in tumour cell proliferation, migration and invasion, and circulating levels of HGF correlate with poor prognosis in hepatocellular carcinoma (HCC). This phase I/II trial (MET111645) is evaluating oral foretinib as first-line therapy in advanced Asian HCC patients.

Methods: Patients with measurable unresectable/metastatic HCC, no prior sorafenib, ECOG PS 0-1, adequate organ function and Child-Pugh score ≤6 are eligible for enrollment. Phase I is a standard 3+3 design using increasing doses of oral foretinib to evaluate safety and determine the maximum tolerated dose (MTD). Secondary objectives include antitumor activity and pharmacokinetics.

Results: A total of 17 patients have been enrolled: median age, 58 (range, 31-78) years, M/F=12/5 BCLC stage 0/A/B/C at screening: 0/0/4/13. Prior lines of therapy including local treatment: unknown/1/2/3/4: 1/5/5/4/2. Drug-related adverse events (AE) were reported in 13 (77%) patients. The most common AEs were hypertension (54%), diarrhoea (31%), thrombocytopenia (23%), peripheral oedema (23%). Serious treatment-emergent toxicities were reported in 54% of patients. Two dose-limiting toxicities (renal failure, proteinuria) were observed at 45 mg OD but no DLTs were observed at 30 mg OD. Eight patients were evaluable for response according to RECIST; three patients had a best response of partial response (PR); 33% objective response rate (ORR). When the tumour response was assessed according to modified RECIST criteria for HCC, one patient showed a complete response and three patients with a PR, 44% ORR.

Conclusion: The MTD was determined to be foretinib 30 mg OD. The early promising signal of activity observed in this phase I needs to be confirmed later in the phase II.

Epigenetic inactivation of the *MIR34B/C* in multiple myeloma

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Introduction and Methods: Epigenetic inactivation of tumour suppressor miRNAs has been implicated in carcinogenesis. We postulated that *MIR34B/C*, a direct transcriptional target of TP53 tumour suppressor protein, might be inactivated by promoter hypermethylation in multiple myeloma (MM), and hence studied methylation of *MIR34B/C* promoter in eight normal marrow controls, eight MM cell lines, 95 diagnostic MM and 23 relapsed MM samples by methylation-specific PCR.

Results: Promoter of *MIR34B/C* was unmethylated in normal controls but homozygously or heterozygously methylated in six (75.0%) MM cell lines, in which *MIR34B/C* expression inversely correlated with the level of *MIR34B* methylation. Moreover, in cell lines harbouring homozygous *MIR34B/C* methylation, treatment with 5-aza-2′-deoxycytidine led to *MIR34B/C* promoter demethylation and *MIR34B* re-expression. Furthermore, restoration of *MIR34B* led to inhibition of cellular proliferation and concomitant increase of apoptosis in MM cells, thereby confirming the tumour suppressive property of *MIR34B/C* in MM. In primary samples, *MIR34B/C* hypermethylation occurred in only 5.3% diagnostic MM but 52.2% relapsed MM samples ($P<0.001$). Moreover, in 12 MM patients with paired samples at both diagnosis and relapse, apart from one showing *MIR34B/C* methylation at both diagnosis and relapse, *MIR34B/C* methylation was acquired at relapse in six (54.5%) patients.

Conclusion: *MIR34B/C* is a tumour suppressor miRNA in MM. Hypermethylation of *MIR34B/C* is tumour-specific with reversible miRNA silencing. Moreover, while methylation of *MIR34B/C* is infrequent at diagnosis, frequent *MIR34B/C* hypermethylation in MM patients at relapse or disease progression implicated a role of *MIR34B/C* hypermethylation in disease progression and accounted for the frequent *MIR34B/C* methylation in cell lines. This is one of the first papers showing the role of miRNA methylation in MM (Blood, in press, 2011).

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88 Endobronchial ultrasound-guided transbronchial needle aspiration in patients presented with superior vena cava syndrome

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Background: Expedient pathological diagnosis is crucial in selection of appropriate treatment in patients presented with superior vena cava syndrome (SVCS). The performance and safety of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) in this setting is unknown.

Methods: Over a 4-year period, patients presented with SVCS in the presence of mediastinal mass and referred for EBUS-TBNA were enrolled for the study. The procedure was performed under local anaesthesia with conscious sedation. TBNA was performed under real-time with the curvilinear probe of EBUS. Rapid on site cytological examination (ROSE) was not available.

Results: Eighteen procedures of EBUS-TBNA were performed in 17 patients. Malignancy was confirmed in 16 patients (diagnostic yield, 94.1%). There was no major complication including significant bleeding or pneumothorax related to the procedures.

Conclusions: EBUS-TBNA has high diagnostic yield and is safe in patients presented with SVCS and mediastinal mass.

89 Serum adiponectin in relation to other obesity-related biomarkers in predicting type 2 diabetes: a 5-year prospective study

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Aims: To identify obesity-related serum biomarkers associated with the development of type 2 diabetes in a Chinese population and to examine if these biomarkers added values to conventional risk factors in diabetes prediction.

Methods: We studied 1315 non-diabetic subjects from the prospective Hong Kong Cardiovascular Risk Factor Prevalence Study (CRISPS). Serum biomarkers including adiponectin, tumour necrosis factor-alpha R2 (TNF-α R2), leptin, adipocyte–fatty acid binding protein (A-FABP) and high-sensitivity C-reactive protein (hsCRP) were measured in baseline samples.

Results: A total of 75 participants developed diabetes over a median of 5.4 years. Low adiponectin, high A-FABP, hsCRP levels were, individually, significantly associated with incident diabetes after adjusting for age, sex, waist circumference (WC), FG, hypertension and dyslipidaemia. A backward stepwise logistic regression model analysing all the significant serum biomarkers showed that hypoadiponectinaemia and high A-FABP were independently associated with incident diabetes: OR=2.525; P=0.019; lowest versus highest quartile, for adiponectin and OR=2.801; P=0.022; highest versus lowest quartile, for A-FABP. Hypoadiponectinemia, but not A-FABP, significantly improved the log-likelihood of a clinical diabetic prediction (CDP) model including sex, age, WC and FG. The ROC curve showed that the CDP + adiponectin model provided a good prediction of diabetes (AUC=0.798; 95% CI, 0.753-0.842).

Conclusions: Our results suggested that serum levels of adiponectin, hsCRP and A-FABP were predictive of the development of type 2 diabetes in Chinese. Hypoadiponectinemia provides the best prediction with added value over traditional risk factors and is of potential use for risk assessment model development.

Diabetes visiting team programme improved glycaemic control of diabetic patients in a regional hospital

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Introduction: Diabetes is a common condition. Many of the diabetic patients are managed by non-diabetologists particularly in regional hospitals. We have started a diabetes visiting team programme to provide regular in- and out-patient consultation services to a regional hospital in Hong Kong West Cluster. The aim of this study was to assess the outcome of this programme.

Methods: Patients referred to the visiting team were seen by a diabetes specialist nurse and an endocrinologist who visited the regional hospital on a weekly basis. Education and advice was given during the on-site consultation. Patients were then followed in nurse-led clinic where intensification of patient education, carer training and ambulatory dosage adjustment were provided as appropriate. Complication screening was arranged as necessary. Stabilised patients were discharged and continued to be followed up by the parent team. Patients who required insulin analogues or multiple insulin injection regimens were referred to diabetes clinic for long-term follow-up. Patients' clinical and metabolic parameters were measured at baseline and 3 months after their initial consultation.

Results: A total of 59 patients were referred over 5 months since programme initiation. They were seen by the team within 21.2 ± 12.4 days after referral. Three months after recruitment to programme, the percentage of patients on home blood sugar monitoring had increased (88.1% vs 47.6% at baseline; P<0.01). Diabetic retinopathy, nephropathy and neuropathy were newly diagnosed in 7, 12 and 4 patients respectively. HbA1c dropped from 10.5 ± 2.1% to 8.2 ± 1.1% (P<0.01). Fasting plasma glucose decreased from 12.4 ± 5.7 to 8.6 ± 2.7 mmol/L (P=0.01). There was an increased usage of DPP-4 inhibitor (17.8% vs 4.4%; P=0.014) and insulin (62.2% vs 42.2%; P=0.007).

Conclusion: This diabetes visiting team programme has been effective in improving glycaemic control of diabetic patients in a regional hospital. It also shows the benefits in detecting undiagnosed diabetic complications and enhancing patient empowerment.

Modulatory effect of mesenchymal stem cells on albumin-induced tubular inflammation

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Background: Bone marrow–derived mesenchymal stem cells (BM-MSCs) have recently shown protective effect in various types of kidney diseases. The mechanism of action, however, is not completely defined. This study aimed to explore the potential impact of BM-MSCs on protein-overloaded PTECs regarding its proinflammatory phenotype.

Methods: PTECs were treated with human albumin serum (HSA) and co-cultured with BM-MSCs for 6 hours and 24 hours. Transcription and secretion of proinflammatory mediators were measured by real-time qPCR and ELISA, respectively. NF-kappaB signalling was assessed by western blot.

Results: BM-MSCs significantly reduced the up-regulated mRNA transcripts and secreted proteins of proinflammatory cytokines (IL6, IL8 and TNF-alpha) and chemokines (CCL2 and CCL5) in PTECs upon HSA challenge (all P<0.05). These effects were associated with attenuation of HSA induced I-kappaB phosphorylation. To dissect the potential mechanism, we detected that the anti-inflammatory genes, HGF and IL1RN, were significantly induced in BM-MSCs during co-culture with PTECs under protein overload condition.

Conclusion: These data suggest an anti-inflammatory role of BM-MSC in HSA-elicited PTECs inflammation, probably through paracrine effects of HGF and IL1RN via NF-kappaB signaling.

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The in-vitro cardioprotective effect of the natural flavone acacetin against ischaemia/reperfusion injury in rat hearts

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Introduction: The natural flavone acacetin from Tianshan Xuelian has been reported to be a promising atrial-selective anti-atrial fibrillation agent. The present study was designed to investigate whether this compound would be cardiac protective against ischaemia/reperfusion insult in isolated rat hearts.

Methods: Isolated rat hearts were retrogradely perfused with a Langendorff apparatus. Electrocardiogram (ECG) and contractile function of the heart were recorded using Powerlab system. After a 30-min equilibrium perfusion, left anterior descending coronary artery was ligated for 30 min, then reperfused by releasing the ligation for 180 min. The hearts were then collected for further analysis.

Results: Ventricular fibrillation (VF) was observed during reperfusion. The incidence of VF was 57% in control group (n=11), and it was reduced to 47%, 23% and 12% in hearts treated with 0.3, 1 and 3 μM acacetin. Left ventricular pressure (LVP) and $+dp/dt_{max}$ were reduced by 74% and 63% at 30 min ischaemia and 63% and 56% after 120 min reperfusion in control group. In 3 μM treatment group, LVP and $+dp/dt_{max}$ were reduced by 56% and 37% at 30 min ischaemia and 46% and 55% after 120 min reperfusion. Myocardial infarction size/area at risk was reduced from 0.6378 of control hearts (n=11) to 0.5587, 0.4021 and 0.2021 with 0.3 (n=9), 1 (n=9) and 3 (n=9) μM acacetin ($P<0.01$ vs control), LDH efflux was calculated as percentage of LDH values measured in the first minute of reperfusion during the same experiment. Release of lactate dehydrogenase (LDH) induced by ischemia/reperfusion was also decreased to 71%, 53% and 28% ($P<0.01$) of control with 0.3, 1 and 3 μM acacetin. Apoptosis assay with terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) revealed that acacetin (3 μM) significantly reduced DNA fragmentation.

Conclusion: These results have demonstrated that acacetin is cardiac protective against ischaemia/reperfusion insult in isolated rat hearts by reducing myocardial apoptosis, suggesting a new therapeutic indication of the natural flavone compound.

Derivation of bone marrow dendritic cells from patients with systemic lupus erythematosus

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Introduction: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease complicated by its multi-system involvement and diverse clinical manifestations. Typically, high titers of type I interferon (IFN) correlating with disease activities are found in the sera of SLE patients. Plasmacytoid dendritic cells (pDCs), being the most potent natural type I IFN-producing cells, are targeted as the key pathogenic factor in SLE. In order to understand how pDCs are involved in the pathogenesis of SLE and whether the abnormalities of pDCs are intrinsic or post-developmental, we compared (1) bone marrow (BM)–derived pDCs (BMDCs) with freshly isolated peripheral pDCs; and (2) the development of BMDCs from patients versus healthy donors.

Methods: Purified $CD34^+$ haematopoietic stem cells from BM were used to generate BMDCs. The culture medium was supplemented with Flt3 ligand (FL) only; FL plus thrombopoietin (TPO); or FL, TPO and mesenchymal stem cell culture supernatant (MSC-sup).

Results: Our preliminary results revealed that BMDCs cultured from both patients and donors supplemented with FL expressed pDC markers BDCA2 and BDCA4 but not CD123. Unlike primary pDCs, these $BDCA4^+$ cells did not respond to nominal pDC stimuli such as CpG or R837. Addition of TPO and MSC-sup supported cell survival and promoted proliferation. Further functional characterisation of these cells will be addressed.

Conclusion: Results in this study draw attention to the effects of TPO and MSC-sup in addition to FL on the development of pDC from BM and suggest BMDCs may not fully represent peripheral pDCs.

Serum immunoglobulin G binding activity to human mesangial cells and its correlation with disease activity in patients with lupus nephritis

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Introduction: Lupus nephritis is characterised by mesangial deposition of immunoglobulins (Ig) which cause glomerular injury. In this study, we examined the binding activity of serum IgG (and its subclasses) to human mesangial cells (HMC) in lupus nephritis patients as well as its correlations with clinical and laboratory parameters.

Methods: Archived serum samples were retrieved from 23 patients with biopsy-proven diffuse proliferative lupus nephritis over a mean follow-up of 74 months. Binding activity (expressed as OD) of total serum IgG and its subclasses (IgG_1, IgG_2, IgG_3, IgG_4) to HMC was ascertained with a cellular ELISA and its correlation with clinical or laboratory parameters examined. Sera from 23 healthy individuals were used as controls.

Results: A total of 189 samples were analysed: 48 samples during active and 141 during inactive disease, defined according to clinical assessment. Binding of serum total IgG to HMC was 0.12 ± 0.09, 0.59 ± 0.37 and 0.74 ± 0.42 OD for healthy controls, inactive lupus, and active lupus respectively (P=0.023 active vs inactive, P<0.001 controls vs active or inactive disease). Binding of serum IgG_1 to HMC was 0.05 ± 0.05, 0.41 ± 0.38 and 0.55 ± 0.40 OD for the three groups respectively (P=0.037 active vs inactive, P<0.001 controls vs active or inactive disease). Controls and lupus patients did not vary in the binding of serum IgG_2, IgG_3 or IgG_4 to HMC. Total IgG and IgG_1 HMC-binding activity correlated with anti-dsDNA levels (r=0.26 and 0.39 respectively, P<0.001 for both), and inversely with C3 levels (r= –0.17 and –0.45 respectively, P<0.05 for both). No correlation was observed between IgG binding to HMC and clinical parameters such as serum creatinine, albumin, or proteinuria. Sensitivity/specificity of total IgG or IgG_1 binding to HMC in the prediction of renal flare was 81.3%/39.7% (ROC AUC=0.61, P=0.03) and 83.8%/41.8% (AUC=0.63, P=0.009) respectively.

Conclusion: There is significant total IgG and IgG_1 mesangial cell-binding activity in the sera of patients with lupus nephritis, especially during active disease, and this binding correlated with the anti-dsDNA antibodies levels.

Survival analysis and causes of death in lupus nephritis patients

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Introduction: This study aimed to define the causes and associated risks of death, compared with the local general population, in Chinese patients with lupus nephritis in the recent era. Such data are prerequisite to refine our treatment strategy for lupus nephritis patients and improve their outcomes.

Methods: The records of all lupus nephritis patients followed in a single centre during 1968-2008 were reviewed. The causes of death were identified, the survival curves constructed, and the standardised mortality ratios (SMR) of potential risk factors were calculated with reference to the local general population.

Results: A total of 230 SLE patients with a history of renal involvement (predominantly Class III/IV lupus nephritis with or without membranous features) were included. The follow-up was 4076.6 person-years (mean, 17.7 ± 8.9 years). Twenty-four (10.4%) patients died, and 85% of the deaths occurred after 10 years of follow-up. The 5-, 10-, and 20-year survival rates were 98.6%, 98.2 % and 90.5% respectively. The leading causes of death were infection (50.0%), cardiovascular disease (20.8%), and malignancy (12.5%). The renal survival rates at 5, 10 and 20 years were 99.5%, 98.0% and 89.7% respectively. The SMR in patients with renal involvement, end-stage renal disease, malignancy, or cardiovascular disease was 5.9, 26.1, 12.9 and 13.6 respectively.

Conclusions: Lupus nephritis is associated with a 6-fold increase in mortality compared with the general population. Lupus patients who develop end-stage renal disease have a 26-fold excess in the risk of death, which is more than twice the risk associated with malignancy or cardiovascular disease in these patients.

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Risk factors and outcome of contamination in patients on peritoneal dialysis: a single centre experience in 15 years

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Objective: Contamination is an important risk factor for peritoneal dialysis (PD)–related peritonitis. This study outlined the clinical characteristics and outcome in PD patients who had touch contamination.

Methods: The case records of PD patients from 1995 to 2010 were reviewed. Patients who had contamination of their PD systems were identified and stratified into the dry and wet contamination group. The risk factors, microbiology and clinical outcomes were compared.

Results: A total of 548 episodes of touch contamination were included (Wet contamination 246; dry contamination 302). Seventeen episodes of peritonitis developed after contamination (3.1%) and all occurred in the wet contamination group (P<0.001). Prophylactic antibiotics significantly reduced the risk of peritonitis (1 out of 182 episodes; P<0.001). Half of the patients had either culture negative or staphylococcal peritonitis and most responded to intraperitoneal antibiotics. Two patients had pseudomonas peritonitis and three had acinetobacter peritonitis, and these patients had less favourable outcome with four required catheter removal.

Conclusion: The overall rate of peritonitis was low after contamination. Wet contamination was associated with a much higher risk of peritonitis. Prophylactic antibiotic after wet contamination was effective in preventing peritonitis.

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Binding activity of serum immunoglobulin G to proximal tubular epithelial cells and its correlation with disease activity in lupus nephritis patients

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Introduction: Lupus nephritis, although characterised by glomerular immunoglobulin deposition, is often associated with tubulo-interstitial inflammation. In this study, we evaluated the serum IgG binding to the proximal tubular epithelial cells (PTEC) and its correlation with disease activity in lupus nephritis patients.

Methods: Archived serum samples were retrieved from 23 patients with biopsy-proven diffuse proliferative lupus nephritis over a mean follow-up of 74 months. Binding activity (expressed as OD) of total serum IgG and its subclasses (IgG_1, IgG_2, IgG_3, IgG_4) to PTEC was ascertained with a cellular ELISA and its correlation with clinical or laboratory parameters examined. Sera from 23 healthy individuals were used as controls.

Results: A total of 189 samples were analysed; 48 samples during active and 141 during inactive disease, defined according to clinical assessment. Binding of serum total IgG to PTEC was 0.34 ± 0.16, 0.62 ± 0.27 and 0.83 ± 0.38 OD for healthy controls, inactive lupus, and active lupus respectively (P=0.001 active vs inactive, P<0.001 active or inactive disease vs control). Binding of serum IgG_1 to PTEC was 0.09 ± 0.05, 0.44 ± 0.34 and 0.71 ± 0.46 OD for the four groups respectively (P=0.002 active vs inactive, P<0.001 active or inactive disease vs control). Controls and lupus patients did not vary in the binding of serum IgG_2, IgG_3 or IgG_4 to PTEC. The total IgG and IgG_1 PTEC-binding activity all correlated with anti-dsDNA levels (r=0.34 and 0.50 respectively, P<0.001 for both), and inversely with C3 levels (r= –0.26 and –0.46 respectively, P<0.002 for both). Sensitivity/specificity of total IgG or IgG_1 binding to PTEC in the prediction of renal flare was 45.8%/80.1% (ROC AUC =.63, P=0.049) and 85.4%/35.5% (AUC=0.63, P=0.045) respectively.

Conclusion: There is significant total IgG and IgG_1 proximal tubular cell-binding activity in the sera of patients with lupus nephritis, especially during active disease, and this binding correlated with the anti-dsDNA antibodies levels.

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Efficacy and tolerability of calcipotriol plus betamethasone dipropionate scalp gel formulation for treatment of scalp psoriasis in Chinese

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Introduction: Scalp is the most common site affected by psoriasis and its involvement can cause physical discomfort and negative psychosocial impact. Therapeutic options for scalp involvement are limited by its tolerability and efficacy. The aim of this study was to investigate the tolerability and clinical efficacy of the two-compound gel formulation after 4 weeks of treatment.

Methods: A prospective study was conducted on 20 patients with moderate scalp psoriasis not on systemic treatment. The efficacy was measured by investigator global assessment (IGA scale, 1-6) and clinical severity score (total sign score, 0-12) . Tolerability and adverse effects were recorded. SF36 was used as a tool to measure the change in quality of life after treatment.

Results: Age of patients ranged from 28 to 76 years. After 2 and 4 weeks of treatment, 10% and 28% achieved 'absent' or 'very mild' by IGA, respectively. 10% and 22% had none, or only one slight, sign of scalp lesions after 2 and 4 weeks of treatment, respectively. The severity score reduced from 4.3 to 2.1 ($P<0.001$). The mean TSS reduced from 7.8 to 2.7 ($P<0.001$). At week 4, clinically meaningful improvements (increase of >5 points) in SF-36 physical (21% reduction) and mental (10% reduction) component summary scores were noted. The gel formulation was well tolerated.

Conclusion: Our findings indicated efficacy as early as 2 weeks with the 2-compound formulation to reduce signs and symptoms of psoriasis with corresponding improvement of quality of life. This formulation is well-tolerated in our locality.

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Myocardial structural alteration and systolic dysfunction in preclinical hypertrophic cardiomyopathy mutation carriers

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Introduction: To evaluate the presence of myocardial structural alterations and subtle myocardial dysfunction during familial screening in asymptomatic mutation carriers without hypertrophic cardiomyopathy (HCM) phenotype.

Methods: Sixteen HCM families with pathogenic mutation were studied and 43 patients with phenotype expression (Mut+/Phen+) and 44 patients without phenotype expression (Mut+/Phen-) were observed. Twenty-five control subjects, matched with the Mut+/Phen- group, were recruited for comparison. Echocardiography was performed to evaluate conventional parameters, myocardial structural alteration by calibrated integrated backscatter (cIBS) and global and segmental longitudinal strain by speckle tracking analysis.

Results: All three groups had similar left ventricular dimensions and ejection fraction. Basal anteroseptal cIBS was the highest in Mut+/Phen+ patients (-14.2 ± 4.6dB; $P<0.01$) and was higher in Mut+/Phen- patients as compared to controls (-16.9 ± 2.8 vs -22.6 ± 2.9dB; $P<0.01$) suggesting significant myocardial structural alteration. Global and basal anteroseptal longitudinal strains ($-9.1 \pm 4.5\%$; $P<0.01$) were the most impaired in Mut+/Phen+ patients as compared to the other two groups. Although global longitudinal strain was similar between Mut+/Phen- group and controls, basal anteroseptal strain was more impaired in Mut+/Phen- patients ($-14.5 \pm 4.1\%$: $P<0.01$) as compared to controls ($-19.9 \pm 2.9\%$; $P<0.01$), suggesting a subclinical segmental systolic dysfunction. A combination of >-19.0dB basal anteroseptal cIBS and >-18.0% basal anteroseptal longitudinal strain had a sensitivity of 100% and a specificity of 74% in differentiating Mut+/Phen- group from controls.

Conclusion: The use of cIBS and segmental longitudinal strain can differentiate HCM Mut+/Phen- patients from controls with important clinical implications for the family screening and follow-up of these patients.

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Impact of glycaemic control on circulating endothelial progenitor cells and arterial stiffness in patients with type 2 diabetes mellitus

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Introduction: Patients with type 2 diabetes mellitus (DM) have increased risk of endothelial dysfunction and arterial stiffness. Levels of circulating endothelial progenitor cells (EPCs) are also reduced in hyperglycaemic states. However, the relationships between glycaemic control, levels of EPCs and arterial stiffness are unknown.

Methods: We measured circulating EPCs and brachial-ankle pulse wave velocity (baPWV) in 234 patients with type 2 DM and compared with 121 age- and sex-matched controls.

Results: Patients with DM had significantly lower circulating LogCD34$^+$/KDR$^+$ and LogCD133/KDR$^+$ EPC counts, and higher LogbaPWV compared with controls (all P<0.05). Among those 120/234 (51%) of DM patients with satisfactory glycaemic control (defined by haemoglobin A1c, HbA1c ≤6.5%), they had significantly higher circulating LogCD34/KDR$^+$ and LogCD133/KDR$^+$ EPC counts, and lower LogbaPWV compared with patients with poor glycaemic control (all P<0.05). The circulating levels of Log CD34/KDR$^+$ EPC (r= –0.46, P<0.001) and LogCD133/KDR$^+$ EPC counts (r= –0.45, P<0.001) were negatively correlated with LogbaPWV. While the level of HbA1c positively correlated with LogbaPWV (r=0.20, P<0.05) and negatively correlated with circulating levels of LogCD34/KDR$^+$ EPC (r= –0.40, P<0.001) and LogCD133/KDR$^+$ EPC (r= –0.41, P<0.001). Multivariate analysis revealed that HbA1c, LogCD34/KDR$^+$ and LogCD133/KDR$^+$ EPC counts were independent predictors of LogbaPWV (P<0.05).

Conclusion: In patients with type 2 DM, the level of circulating EPCs and arterial stiffness were closely related to their glycaemic control. DM patients with satisfactory glyacemic control had higher levels of circulating EPCs and were associated with lower arterial stiffness.

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BK_{Ca} and hEAG channels modulate proliferation and differentiation of human bone marrow–derived mesenchymal stem cells

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Introduction: Bone marrow–derived mesenchymal stem cells (MSCs) are a promising cell source for regenerative medicine. However, cellular physiology is not fully understood in human MSCs. The present study was to determine the potential role of the dominant functional ion channels, large-conductance Ca^{2+}-activated potassium (BK_{Ca}) channel, ether-à-go-go potassium (hEAG1) channel in regulating cell functions, including proliferation and differentiation, in human MSCs.

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 flowcytometry. Lentivirus-based shRNA was used to knock down ion channels specifically. RT-PCR and Western blot analysis were applied. Adipogenic differentiation was visualised by Oil red O staining. Osteogenic differentiation was determined by alizarin red S staining.

Results: We found that paxilline and astemizole respectively reduced BK_{Ca} and hEAG1 current in human MSCs. The cell proliferation assay with MTT and ^{3}H-thymidine incorporation methods revealed that the inhibition of BK_{Ca} with paxilline and hEAG1 with astemizole decreased cell proliferation and reduced DNA synthesis rate in a dose-dependent manner. Flowcytometry analysis displayed that paxilline and astemizole accumulated human MSCs at G0/G1 phase, and decreased cell population of S phase. Moreover, lentivirus-based shRNAs targeted to BKCa or hEAG1 channel remarkably reduced both mRNA and protein expression of BK_{Ca} or hEAG1 channel; and proliferation of human MSCs was reduced by BK_{Ca}-shRNAs or hEAG1-shRNAs. We also found these effects were accompanied by a decreased expression of cyclin D1 and cyclin E. In addition, we found that knock-down of BK_{Ca} or hEAG1 channels reduced differentiation ability of hMSCs. The expression level of PPARγ or osteocalcin was decreased after the knock-down of KCNH1 or KCNMA1 respectively when hMSCs were induced to adipogenic or osteogenic differentiation.

Conclusion: Our results demonstrate that BK_{Ca} and hEAG1 channels participate in the regulation of cell proliferation by promoting G0/G1 cells into cell cycling progression, and are also closely involved in cell differentiation in human MSCs.

In-vitro HCN2 expression and functional significance in small-cell lung cancer

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Introduction: Small-cell lung cancer (SCLC) is a form of neuroendocrine tumour that often over-expresses various neuropeptides. HCN2 (hyperpolarization-activated cation channel) is a novel ion channel that plays an important functional role in cardiac conducting tissues and possibly in nerve tissues. We hypothesised that HCN2 would be expressed in SCLC and played an important functional role in proliferation or survival.

Methods: Our in-vitro cell line model consisted of one normal bronchial epithelia cell (BEAS-2B), three SCLC (H187, DMS79, H1668), and eight non-small cell lung cancer (NSCLC) cell lines (H1975, H358, H23, H827, H1734, H 2935, H4006, H1650) from ATCC. Q-PCR was used to detect HCN2 gene expression. MTT assay was used to quantify proliferation. Specific HCN2 inhibitor (ZD7288) was used to determine the effect of HCN2 blockade on cell proliferation. HCN2 protein expression in human SCLC tissue was examined by immunohistochemistry (IHC).

Results: HCN2 was found to be expressed in most lung cancer cell lines, preferentially over-expressed in SCLC compared to NSCLC cell lines. Functional blockade of the inward current on cell membrane with ZD7288 (100 μM) resulted in significant cell death especially in SCLC compared with NSCLC and BEAS-2B, which correlated with HCN2 expression levels (r=0.85). In a human SCLC specimen, IHC demonstrated cytoplasmic staining in tumour cells, which is even stronger than the control with human hippocampal brain tissues.

Conclusions: HCN2 is a novel ion channel that is over-expressed in SCLC in vitro. The impact of HCN2 pharmacological blockade on cell survival suggests an important functional role of HCN2 in SCLC. Ongoing studies are carried out to confirm this phenomenon and delineate the mechanisms on survival pathways.

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Arsenic trioxide mediates cell death via loss of mitochondrial trans-membrane potential through oxidative stress in small-cell lung cancer

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Introduction: Arsenic trioxide (ATO) has been clinically used to treat acute promyelocytic leukaemia with high chance of complete remission. In-vitro studies have also suggested effects of ATO on apoptosis and regulation of biological functions in other solid cancers, with mechanisms that have not been completely elucidated. In this study, we investigated the effect of ATO on cell proliferation in a small-cell lung cancer (SCLC) cell line model (H841) and its underlying mechanisms.

Methods: Drug treatment (ATO) experiments were performed in a SCLC cell line H841 (an adhesion line) 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 cells undergoing apoptosis was detected by annexin-V/7-AAD assay with flow cytometry. Mitochondrial depolarization was determined by JC-1 staining. The production of reactive oxygen mediators was examined by 2′,7′-dichlorodihydrofluorescein diacetate (H2DCF-DA). Glutathione (GSH) content was detected by CM-FDA. Western blotting was performed for apoptotic factors (cleaved caspase-3, cleaved caspase-8, cytochrome c, AIF).

Results: ATO induced cell death and loss of mitochondrial trans-membrane potential in a dose-dependent manner with down-regulation of Bcl-2, up-regulation of cleaved caspase 3/8, and mitochondrial release of cytochrome c and AIF. Pan-caspase inhibitor (V-ZAD-FMK) could alleviate cell death (up to 20% decrease) and mitochondrial depolarization (around 10-15% decrease) induced by ATO. Reactive oxygen species (ROS) was increased upon treatment with ATO. Concurrent treatment with antioxidant N-acetylcysteine (NAC) and ATO resulted in significant reduction of apoptosis (30-60% vs 5-10%), mitochondrial depolarization (30-50% vs 5-10%), and ROS production compared with single treatment with ATO. However, ATO-induced GSH depletion was not affected by co-treatment with NAC.

Conclusion: ATO mediates cell death mainly through apoptosis via both caspase dependent and independent pathways in SCLC. Furthermore, mitochondrial depolarization initiated by oxidative stress plays a key role in the action of ATO in SCLC.

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