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20th Medical Research Conference, 17 January 2015

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The AAV toolkit for gene therapy and basic science applications

IE Alexander

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Massively parallel sequencing technology is driving an unprecedented explosion in genetic knowledge with immense implications for basic science and human health. The most immediate and readily achievable clinical impact is increased diagnostic power. Realising therapeutic potential, however, is markedly more challenging and demands ongoing development of genomic technologies. Particularly exciting is the progress being made in the gene therapy field which is underpinned by the evolution of gene transfer and genome editing technologies. Recombinant vectors based on adeno-associated virus (AAV) are an excellent exemplar. AAV-based vectors are not only powerful tools for gene transfer, but can also be deployed to achieve targeted genome editing. This presentation will explore the rapidly evolving AAV vector toolkit, examining its utility as a research tool and highlighting current use in human gene therapy trials. Particular emphasis will be given to the exciting prospect of achieving therapeutic benefit by targeted genome editing both in vitro and in vivo.

Drugs developed to treat type 2 diabetes show protective effects in Parkinson's and Alzheimer's disease

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Long-acting analogues of the incretin hormone glucagon-like peptide-1 (GLP-1) have shown very promising results in preclinical studies of a range of diseases such as Alzheimer's disease (AD) and Parkinson's disease.¹ The underlying mechanisms are the growth-factor properties of GLP-1 to protect from oxidative stress, induce cell repair, promote stem cell activation, reduce apoptosis, and promote synaptogenesis and synaptic activity. GLP-1 analogues can readily cross the blood brain barrier, which set them apart from other growth factors.¹ Another important aspect is that there are GLP-1 receptor agonists already on the market as a treatment for type 2 diabetes: liraglutide (Victoza[®]), exendin-4 (Byetta[®]), lixisenatide (Lyxumia[®]).

We have tested a range of these drugs in a transgenic mouse model of AD. Both liraglutide and lixisenatide were effective in reducing the hallmarks of AD in APP/PS1 mice. We tested the drugs in 9-month-old mice, an age when they start to develop impairments. The beta-amyloid plaque load, total amyloid levels, synapse loss, oxidative stress, and the chronic inflammation response were reduced in the brain of these mice after once-daily intraperitoneal injections for 8 weeks. Memory formation was protected by the drug and synaptic plasticity in the hippocampus was enhanced, demonstrating that not only was synapse loss prevented, but that the synapses are functioning very well. In addition, neuroprogenitor proliferation and neurogenesis in the dentate gyrus was normalised.¹ We also tested liraglutide in 14-month-old mice to test if the drug may be helpful even in advanced stages of AD. We found that even at that stage, liraglutide still was able to improve on some of the biomarkers of AD.² The novel GLP-1 mimetic lixisenatide (Lyxumia[®]) also showed good neuroprotective effects in this mouse model.³

Based on the extensive pre-clinical evidence, several clinical trials are currently under way, testing liraglutide and exendin-4 (Byetta) in AD and Parkinson's patients. We have started a clinical trial of liraglutide in AD patients. A recently completed clinical trial of exendin-4 in Parkinson's patients showed very promising effects, including a clear improvement in the Mattis dementia rating scale. Further clinical trials of liraglutide in Parkinson's patients are planned.

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Spatial and temporal dynamics of cancer evolution

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Increasing evidence supports complex sub-clonal relationships in solid tumours, manifested as intratumour heterogeneity. Our group and others are finding evidence for spatial heterogeneity within individual tumours and the temporal dynamics of tumour evolution. Parallel evolution of sub clones, with distinct somatic events occurring in the same gene, signal transduction pathway or protein complex, suggests constraints to tumour evolution that might be therapeutically exploitable. Drivers of tumour heterogeneity appear to change during the disease course that contribute to the temporally distinct origins of cancer driver events. Genome doubling, occurring early or late in tumour evolution, exacerbates chromosomal instability contributing to intercellular heterogeneity and poor outcome. The finding of sub-clonal driver events is likely to limit the efficacy of targeted monotherapies, suggesting the need for new approaches to drug development and clinical trial design. TRACERx, a longitudinal lung cancer evolution study and DARWIN clinical trials aimed at deciphering the relevance of sub-clonal driver events to therapeutic outcome, will be discussed.

Role of autophagy in vertebrate haematopoiesis and human myelodysplastic syndrome

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Introduction: Autophagy is a conserved cellular process essential for the maintenance of cytoplasm and clearance of expired proteins and organelles, which is upregulated during important cellular events, for examples, mitosis and differentiation. Haematopoiesis is such a process where haematopoietic stem cells actively undergo mitotic division and differentiation for its lifelong maintenance of all blood cell types. Myelodysplastic syndrome (MDS) is a clonal bone marrow disease characterised by insufficient haematopoiesis leading to cytopenias. Cellular consequences of defective autophagy, for examples, damaged mitochondria and increased apoptosis, have been observed in MDS patient samples, and may imply that dysregulation of autophagy contributes to the pathogenesis of MDS. However, functional study about autophagy-related genes are lacking and the precise role of autophagy in vertebrate haematopoiesis remains unknown. With a principal focus on the genes *ATG13* which forms the adaptor protein essential for the autophagy initiation complex, the present study aimed to make use of the zebrafish model to examine the role of autophagy and its dysregulation in haematopoiesis.

Methods: Expression pattern of zebrafish *atg13* was examined by whole-mount in-situ hybridisation (WISH) and semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR). To knock-down *atg13*, anti-sense morpholino (MO) targeting start codon of *atg13* was injected into zebrafish embryos and the effect was evaluated by WISH and quantitative RT-PCR (Q-PCR). Molecular targeting of *atg13* MO was tested with an *atg13*-EGFP (enhanced green fluorescent protein) chimeric reporter.

Results: Zebrafish *atg13* expressed throughout embryonic development. It expressed ubiquitously up to 24 hpf (hours post-fertilisation) and was more confined to the encephalic region afterward. MO-mediated knock-down of *atg13* did not demonstrate any observable phenotypes at dosages of up to 6 ng and cardiac oedema was observed at high injection dosages (≥ 9 ng). Molecular targeting of *atg13* MO was confirmed by the quenching of green fluorescent protein expression in embryos co-injected with the *atg13*-EGFP chimeric reporter. WISH and Q-PCR suggested that *atg13* MO injected at 6 ng has no effect on various haematopoietic lineages.

Conclusion: Knock-down of *atg13* alone does not induce significant haematopoietic defects in the zebrafish model, albeit expressed at detectable levels throughout its embryonic development. Further work will be required to confirm its role and explore other target genes involved in autophagy.

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The usefulness of diffusion-weighted imaging in detecting spinal disease activity in spondyloarthritis patients

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Introduction: To compare the two magnetic resonance imaging (MRI) sequences, the diffusion-weighted imaging (DWI) and short-tau inversion recovery (STIR) in assessing the spine and sacroiliac (SI) joints disease activities in spondyloarthritis (SpA) patients.

Methods: Overall, 31 consecutive SpA patients with back pain were included in the analyses. DWI and STIR MRI were simultaneously performed. Activities were scored using the SpondyloArthritis Research Consortium of Canada (SPARCC) spine and SI joints MRI indices for ankylosing spondylitis. Results were compared with each other, with back pain Numerical Rating Score (NRS), clinical parameters, and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI). Correlation coefficients were also determined between SPARCC scores of DWI and STIR images.

Results: Patients with inflammatory back pain or back pain of NRS ≥ 4 had significantly more STIR-detected (87.5% vs 12.5%, $P=0.004$; 80.0% vs 20.0%, $P=0.04$) and DWI-detected (81.2% vs 12.8%, $P=0.02$; 75.0% vs 25.0%, $P=0.08$) bone marrow oedema. Both the back pain NRS of ≥ 4 and BASDAI of ≥ 4 groups showed no correlation with SPARCC of STIR spine (odds ratio [OR]=1.05, $P=0.62$; OR=0.91, $P=0.21$), STIR SI joints (OR=0.95, $P=0.31$; OR=0.93, $P=0.24$); and DWI spine (OR=0.95, $P=0.43$; OR=0.91, $P=0.13$), DWI SI joints (OR=0.84, $P=0.33$; OR=0.85, $P=0.43$). The DWI spine SPARCC scores correlated strongly with STIR spine SPARCC scores (OR=0.72, $P<0.001$) while moderate correlation was found at SI joints level (OR=0.47, $P=0.02$).

Conclusion: DWI-detected bone marrow oedema correlates well with that of the STIR image. Presence of bone marrow oedema in MRI is an additional information and needs to be interpreted with clinical parameters.

Local adverse effect of intradermal administration of influenza vaccination is an indicator of satisfactory immunogenicity

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Background: There are few studies concerning the association between immunogenicity of intradermal (ID) administration of influenza vaccination and adverse effect of vaccination.

Methods: It was a subgroup analysis of ID vaccination recipient of a randomised controlled trial comparing ID and intramuscular influenza vaccination. Outcomes were immunogenicity (in terms of seroconversion of H1N1 strain at day 21) and short-term (within 7 days) adverse effects. Adverse effects were divided into local (like swelling and redness) and systemic (like fever and myalgia).

Results: Overall, 50 nursing home older adults had received ID vaccination. At day 7, 30 of them had satisfactory immunogenicity (seroconversion with ≥ 4 -fold increase in antibody titre). Of them, 13 had one or more kinds of local adverse effect, with redness being the most common; 8 of them had one or more kinds of systemic adverse effect, with malaise being the most common. All participants with any local adverse effect had satisfactory immunogenicity. There was significant association between any local adverse effect and satisfactory immunogenicity ($P=0.002$). There was no significant association between any systemic adverse effect and satisfactory immunogenicity ($P=0.44$).

Conclusion: Local adverse effect of ID administration of influenza vaccination is an indicator of satisfactory immunogenicity.

Comparing effectiveness between intramuscular and intradermal trivalent influenza vaccination in nursing home residents: a randomised controlled trial

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Background: There is no study comparing clinical effectiveness between intradermal (ID) and intramuscular (IM) administration of influenza vaccination in nursing home older adults.

Methods: This was a single-centre, randomised, controlled, open-label, parallel group trial from October 2013 to April 2014 in nine nursing homes comparing the effectiveness between full-dose IM and ID immunisation of the trivalent influenza vaccine. Outcomes were 1-year hospitalisation due to pneumonia and 1-year laboratory-confirmed influenza infection between the two groups.

Results: Overall, 100 nursing home older adults (mean age, 82.9 ± 7.4 years; male, 36%) were randomised. Baseline characteristics were similar between the two groups. At 1 year, 8 and 11 participants were hospitalised at least once due to pneumonia in ID and IM groups, respectively ($P=0.44$). Two and four participants had laboratory-confirmed influenza infection in ID and IM group, respectively ($P=0.68$).

Conclusion: ID vaccination of influenza vaccine may not be more effective than IM vaccination. However, this study was limited by its small sample size and both clinical outcomes were lower in ID group. Clinical trial with larger sample size would be necessary.

A randomised controlled trial to compare immunogenicity between intramuscular and intradermal trivalent influenza vaccination in nursing home older adults

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Background: Immunosenescence in older adults contributes to unsatisfactory immunogenicity towards influenza vaccine. Intradermal (ID) administration of influenza vaccine has been suggested to improve immunogenicity but there is no study regarding the immunogenicity of ID influenza vaccination in nursing home older adults.

Methods: This was a single-centre, randomised, controlled, open-label, parallel group trial from October 2013 to April 2014 in nine nursing homes comparing the immunogenicity and safety between full-dose intramuscular (IM) and ID immunisation of the trivalent influenza vaccine. Day-21 and day-180 immunogenicity of ID compared to IM vaccination was analysed.

Results: Overall, 100 nursing home older adults (mean age, 82.9 ± 7.4 years; male, 36%) were randomised. Baseline characteristics were similar between the two groups. At day 21, non-inferiority in immunogenicity of the ID vaccination was demonstrated. The seroconversion rate of the H1N1 strain was significantly higher in the ID group. At day 180, immunogenicity of both groups fell but the geometric mean titre (GMT) of all strains in the ID group was higher and the difference was significant for H3N2 strain. The seroconversion rate and GMT fold increase of H3N2 strain was significantly higher in the ID group.

Conclusion: ID vaccination of influenza vaccine is non-inferior to IM vaccination in immunogenicity in nursing home older adults. Furthermore, ID vaccination is superior in some components of the immunogenicity assessment.

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Prevalence of influenza vaccination and associated factors among Chinese nursing home health care workers

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Background: Influenza vaccination (IV) in nursing home health care workers (nHCWs) can reduce mortality of nursing home older adults. There is no study examining the prevalence of IV in Chinese nHCWs.

Methods: Self-reported anonymous questionnaires based on Health Belief Model were distributed to nHCWs of 58 Hong Kong nursing homes.

Results: A total of 1398 questionnaires were distributed and 1300 were returned (response rate, 93%). Of all respondents, 55.9% (n=727) received IV; 37.6% (n=489) of respondents believed IV was "non-efficacious" or "don't know" about efficacy, and 41.2% (n=536) of respondents perceived side-effects of IV to be "severe/moderate" or "don't know" about side-effects. Multivariate analysis showed that nHCWs were less likely than others to receive IV if they (i) perceived IV as "non-efficacious", (ii) "don't know" about efficacy, (iii) perceived side-effects as "severe/moderate", (iv) "don't know" about side-effects, (v) perceived influenza infection as "mild/not severe", (vi) "don't know" about severity of influenza infection, (vii) were not worry of contracting influenza in the coming 12 months, and (viii) had never vaccinated in the past. On the other hand, nHCWs who aged 50 to 59 years and aged ≥ 60 years were more likely than others to receive IV.

Conclusion: The prevalence of IV among Chinese nHCWs was 55.9%. Inadequate knowledge regarding the efficacy and side-effects of IV were major barriers of receiving IV. Enhanced promotion strategies on IV should be implemented to address the knowledge gap and improve the vaccination rate.

Epidemiology of invasive fungal disease in haematology patients: a two-year prospective analysis

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Introduction: Invasive fungal diseases (IFDs) are major complications during treatment of haematological malignancies, and are associated with significant morbidity and mortality. Recipients of haematopoietic stem cell transplant and induction chemotherapy for acute leukaemia are particularly at risk of developing such complications. Both invasive mould and yeast infection can occur. Prophylactic antifungal could potentially reduce the incidence and hence mortality associated with IFD, but optimal regimen relies on knowledge of local prevalence of IFD. We have conducted a 2-year prospective study on the epidemiology of IFD in haematology patients.

Methods: Consecutive patients who developed IFDs during treatment of haematological malignancies were enrolled from June 2012 to June 2014. Baseline characteristics, underlying haematological disease and treatment, type of fungal organisms involved, prophylaxis and treatment used, and 30-day mortality were recorded.

Results: Overall, 31 patients developed IFDs during treatment of haematological malignancy. The median age was 48 years. Acute myeloid leukaemia (AML) represented the largest proportion of underlying haematological and majority of the patients with AML were receiving third-line induction treatment during the occurrence of IFD. *Candida* and *Aspergillus* accounted for the majority of IFD, seconded by *Cryptococcus neoformans*.

Conclusions: This study represents a snapshot of our local prevalence of IFD in our patient cohort, a pattern similar to that of other western countries. Increased use of echinocandin prophylaxis may account for the increase of cryptococcal infection.

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APPL1 counteracts streptozotocin-induced diabetes in mice

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Introduction: The adapter protein APPL1 has been reported for its beneficial role against hyperglycaemia in type 2 diabetes by promoting both secretion and actions of insulin. In this study, we aimed to investigate whether APPL1 plays a protective role in β -cell function in type 1 diabetes.

Methods: Both 12-week-old male APPL1 knockout (KO) mice and their wild-type (WT) littermates were treated with 50 mg/kg streptozotocin (STZ), a glucosamine-nitrosourea compound that induced β -cell apoptosis for 5 consecutive days. Fed glucose levels were monitored for 10 days after last injection and glucose tolerance test was performed 5 days after last injection. Pancreases were collected after the mice were sacrificed. Haematoxylin & Eosin staining was performed to study morphological changes. Insulin content and autophagy marker p62 were detected by immunohistochemical (IHC) staining. Pancreatic β -cell apoptosis was revealed by TUNEL assay.

Results: APPL1 KO mice displayed higher fed glucose levels and glucose intolerance when compared with their WT littermates after STZ injection. Such changes were due to defective glucose-stimulated insulin secretion and reduced insulin content in pancreas and β -cell mass. In addition, APPL1 KO mice exhibited increased β -cell apoptosis. IHC staining revealed that expression of p62 was elevated in β -cells of APPL1 KO mice, indicating that this protective pathway was impaired.

Conclusion: We conclude that APPL1 counteracts STZ-induced diabetes by protecting pancreatic β -cell from apoptosis, and will examine whether such protective action is mediated by autophagy in the future study.

Acknowledgement

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Identification of polo-like kinase as a potential target in acute myeloid leukaemia

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Internal tandem duplication (ITD) in *fms*-like tyrosine kinase 3 (FLT3) is the commonest mutation (approximately 30%) in acute myeloid leukaemia (AML). FLT3 mutation confers poor prognosis when treated with conventional chemotherapy and allogeneic haematopoietic stem cell transplantation. In spite of the effectiveness of tyrosine kinase inhibitor (TKI) in targeting FLT3-ITD+ AML, emergence of tyrosine kinase domain mutation in FLT3 and changes in gene expression have been reported to be critical in the acquisition of TKI resistance after treatment. Previously, we have reported that cell cycle regulators including polo-like kinase 1 (PLK1), cell division cycle 25 homolog A (CDC25A), cyclin B2 (CCNB2), and cyclin E1 (CCNE1) were up-regulated in sorafenib-resistant AML. In particular, PLK1 regulates several checkpoints in cell cycle. We hypothesised that aberrant cell cycle progression mediated by PLK1 confers survival advantage to AML, particularly in drug-resistant AML, thus targeting PLK1 could be a potential therapeutic strategy.

In-vitro treatment with PLK1 inhibitors significantly inhibited the growth of AML cell lines (MOLM-13, MV4-11, KG-1, ML2, Kasumi-1, NB4, THP-1, and OCI-AML3) with IC50 ranging from 35.1 to 98.4 nM. The growth inhibitory effects of PLK1 inhibition on FLT3-ITD+ cell lines correlated with induction of apoptosis and cell cycle arrest at G2/M phase. Furthermore, both PLK1 inhibitors significantly suppressed the growth of sorafenib-resistant cell line and primary AML samples.

We conclude that PLK1 could be an important target in AML and targeting PLK1 might be an effective approach in targeting drug-resistant AML.

Evaluation of various cutpoints for low lean mass and slow gait speed in predicting death in the National Health and Nutrition Examination Survey 1999-2004

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Introduction: Sarcopenia is commonly defined as loss of muscle mass with limited muscle function or strength. Various cutpoints of low lean mass and slow gait speed have been proposed by different professional working groups. We compared the performance of various cutpoints of low lean mass and slow gait speed in predicting death.

Methods: We analysed data from the continuous National Health and Nutrition Examination Survey conducted between 1999 and 2004, and the subsequent follow-up data on mortality up to 31 December 2006. For low lean mass, cutpoints based on appendicular lean mass (ALM) alone, ALM adjusted for body mass index (ALMBMI), and ALM adjusted for height squared (ALMH2) were evaluated. For slow gait speed, the cutpoints based on 0.7 m/s, 0.8 m/s, 0.9 m/s, and 1.0 m/s were evaluated. A Cox-proportional hazard regression model with adjustment for multiple confounding factors was used for the association analyses.

Results: For low lean mass, the cutpoints based on ALMBMI (<0.512 in women and <0.789 in men) showed the most significant association and highest hazard ratio (HR) with death (HR=1.61; 95% confidence interval [CI], 1.18-2.19; P=0.003). For slow gait speed, all cutpoints tested showed significant association with death in the full model (P<0.001), while the cutpoint of 0.8 m/s showed the highest HR (HR=2.27; 95% CI, 1.54-3.35).

Conclusions: Low lean mass defined by ALMBMI showed the strongest association with death; while slow gait speed (cutpoints ranging from 0.7 m/s to 1.0 m/s) showed significant association with death, with the strongest association being observed for the cutpoint of 0.8 m/s. Further studies validating the cutpoints are warranted before using them in clinical settings.

People with peripheral arterial disease and diabetes are associated with clinically significant weakness and mobility impairment

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Introduction: Low lean mass is associated with weakness, limited mobility, and increased risk of mortality. In the recent effort of Foundation for the National Institutes of Health (FNIH) Sarcopenia Project, an appendicular lean mass (ALM) cutpoints for clinically significant weakness were derived. Peripheral arterial diseases (PAD) and diabetes have been shown to be associated with reduced lean mass. However, whether these diseases are associated with clinically significant weakness remain unknown. In the current study, we aimed to investigate whether PAD and diabetes are associated with clinically significant weakness and mobility impairment as defined by the FNIH Sarcopenia Project.

Methods: Data on 4841 participants aged ≥ 40 years of the National Health and Nutrition Examination Survey 1999-2004 were examined. ALM was measured using dual-energy X-ray absorptiometry. Logistic regression was used to assess the association of diabetes and PAD with low lean mass. Low lean mass was defined as ALM of <19.75 kg in men and ALM of <15.02 kg in women.

Results: In the simple model adjusted for age, sex, body mass index, and race/ethnicity, participants with PAD alone and both PAD and diabetes were associated with low lean mass with an odds ratio (OR) of 1.65 (95% confidence interval [CI], 1.07-2.56) and 2.07 (95% CI, 1.10-3.89), respectively. No association was observed between diabetes and low lean mass. After further adjustment for smoking, drinking, exercise, hypertension, estimated glomerular filtration rate, microalbuminuria, serum biomarkers of liver function, and cardiometabolic biomarkers, participants with both PAD and diabetes remained significantly associated with low lean mass (OR=2.08; 95% CI, 1.03-4.19). No association was observed for diabetes only and PAD only with low lean mass.

Conclusions: People with both diabetes and PAD had a higher likelihood of low lean mass and hence clinically significant weakness. Intervention to improve muscle mass and strength may be useful to improve mobility and reduce risk of mortality in these people.

Non-invasive score identifies ultrasonography-diagnosed non-alcoholic fatty liver disease and predicts mortality in the United States

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Background: Several non-invasive prediction scores for non-alcoholic fatty liver disease (NAFLD) have been developed, but their performance has not been compared and validated in the same population, and whether these prediction scores can predict clinical outcomes remains unknown. In this study, we aimed to validate and compare the performance of four NAFLD prediction scores: fatty liver index, hepatic steatosis index, lipid accumulation product, and NAFLD liver fat score (LFS); and to evaluate the ability of the best NAFLD prediction score to predict mortality.

Methods: We analysed data from the National Health and Nutrition Examination Survey conducted in 1988 to 1994, and subsequent follow-up data for mortality up to 31 December 2006. NAFLD was defined by ultrasonographic detection of hepatic steatosis in the absence of other known liver diseases.

Results: In a group of 5184 participants, LFS consistently showed the highest area under the curve for predicting the presence of NAFLD. During a median follow-up of 14.7 years (range 0.1-18.2 years) and 83 830.5 person-years, participants in the high LFS group ($LFS \geq 1.257$) had a higher cardiovascular and liver-related mortality than participants in the low ($LFS \leq -1.413$; cardiovascular hazard ratio [HR]=2.24, 95% confidence interval [CI]=1.03-4.88; liver HR=31.25, 95% CI=3.13-333.33) or intermediate ($-1.413 < LFS < 1.257$; cardiovascular HR=2.3, 95% CI=1.19-4.48; liver HR=30.3, 95% CI=4-250) LFS groups in the fully adjusted model. Similar results were obtained when LFS was treated as a continuous variable.

Conclusions: LFS is the best non-invasive prediction score for NAFLD, and people with a high LFS score have an increased risk for cardiovascular and liver-related mortality.

Serum calcium and incident diabetes: a retrospective study in Hong Kong Chinese and a meta-analysis

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Background: Serum calcium and its metabolism play an important role in glucose metabolism, whether serum calcium and calcium intake can predict diabetes remains largely unknown. We aimed to evaluate the association of serum calcium with incident diabetes.

Methods: We conducted a retrospective cohort study on 1702 male and 4394 female Southern Chinese aged 20 years or above free of diabetes at baseline. We also searched PubMed, MEDLINE, and Cochrane library in September 2014 to identify observational studies accessing the association between elevated serum calcium and incident diabetes. The overall relative risks (RRs) were calculated using fixed-effect model with inverse variance method.

Results: In 59 130.9 person-years of follow-up, 631 participants developed diabetes. Serum total calcium (third quartile: hazard ratio [HR]=1.42; 95% confidence interval [CI], 1.12-1.8; highest quartile: HR=1.42; 95% CI, 1.11-1.79; as compared to the lowest quartile) was significantly associated with incident diabetes. Addition of serum total calcium to age, sex, and body mass index (BMI) significantly improved integrated discrimination and category-less net reclassification index. Significant interactions with BMI and age were observed. Greater total calcium intake was significantly associated with lower incident diabetes (comparing extreme quartile, HR=0.78; 95% CI, 0.61-0.98). In meta-analysis, two studies together with our results were included, the total participants were 34 117 and the pooled RR was 1.43 (95% CI, 1.20-1.70) comparing individuals of high serum calcium level who had incident diabetes to those who did not have.

Conclusions: Elevated serum total calcium and probably lower total calcium intake were associated with incident diabetes. Adding serum total calcium to basic clinical risk factors significantly improved risk prediction. The mechanism warrants further investigation.

Association between arsenic and diabetes mellitus in National Health and Nutrition Examination Survey 2007-8

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Objective: Whether low-level exposure to arsenic in the environment is associated with diabetes is controversial. We therefore studied this association in the National Health and Nutrition Examination Survey (NHANES) 2007-8.

Methods: In NHANES 2007-8, urine arsenic was measured in around one third of participants. After excluding children and those with missing fasting blood glucose or urine arsenic data, 591 participants were included in the analysis. There were 109 (18.4%) participants with diabetes, defined as glycosylated haemoglobin of $\geq 6.5\%$, fasting serum glucose of ≥ 126 mg/dL, self-reported previous physician diagnosis of diabetes, or self-reported use of anti-diabetic medication.

Results: Comparing the highest and lowest quintiles of total urine arsenic (>13.94 vs 3.38 $\mu\text{g/L}$), the odds ratio for diabetes was 1.71 (0.78-3.72) in the unadjusted model, but after adjustment for age, sex, ethnicity, education, body mass index, cotinine, blood mercury, urine creatinine and anti-hypertensive medication use, the odds ratio was 3.22 (1.21-8.56) [$P=0.022$]. The result remained significant after subtracting arsenobetaine concentration. There was a weak relationship between fasting blood glucose and urinary total arsenic ($r=0.07$, $P=0.035$).

Conclusions: High arsenic exposure (total urine arsenic >13.94 $\mu\text{g/L}$) in one fifth of Americans is associated with diabetes, and fasting blood glucose is weakly related to urinary arsenic. Our results do not prove a causal link between arsenic and diabetes. However, as arsenic is present in drinking water and foods such as rice, it would be prudent to monitor the exposure of the general population to arsenic and keep it low.

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Objectives: Mercury is an environmental hazard. Therefore, we studied recent trends in the blood level of organic and inorganic mercury in the United States.

Methods: We analysed newly available data on blood inorganic mercury levels in the United States National Health and Nutrition Examination Survey (NHANES) 2005-2010. Organic mercury level was calculated by subtracting inorganic mercury level from the total mercury level. As complex sampling was used in NHANES, appropriate weights were used to adjust for oversampling of minorities and sampling from the same location.

Results: There were 8364, 8161, and 8727 participants in NHANES 2005-6, 2007-8, and 2009-10, respectively. Inorganic mercury levels (geometric mean [95% confidence interval]) were 0.31 [0.30-0.32], 0.30 [0.30-0.31], 0.28 [0.27-0.28] µg/L and organic mercury levels were 0.24 [0.19-0.30], 0.19 [0.14-0.25], 0.27 [0.22-0.33] µg/L in 2005-6, in 2007-8, and in 2009-10, respectively. Inorganic mercury levels showed a significant decreasing trend ($P < 0.05$). Organic mercury levels were significantly lower in participants aged < 20 years compared to those ≥ 20 years. The adjusted proportion (mean \pm standard error) of participants with a total mercury level of ≥ 5.8 µg/L was $3.0 \pm 0.2\%$, $3.5 \pm 0.6\%$, and $4.0 \pm 0.4\%$ ($P < 0.05$) in NHANES 2005-6, 2007-8, and 2009-10, respectively.

Conclusions: Inorganic mercury level has been decreasing during the study period. Organic mercury level was lower in 2007-2008 but increased in 2009-10. The significant increase in organic mercury level in the US general population in 2009-10 is of concern, suggesting that continual monitoring of mercury levels is needed.

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Objective: Clinic blood pressure (BP) is only an approximate measure of the true blood pressure. It tends to decrease towards the true value on repeated measurements. The aim of this analysis was to identify the factors related to the change in systolic and diastolic BP on repeated measurements.

Methods: Data on BP and other clinical characteristics were obtained from the National Health and Nutrition Examination Survey (NHANES) database. In 2007-8, there were 4943 participants aged 20 years or over with repeated BP measurements. Repeated measurements analysis of variance was used to identify variables related to the change in BP with time.

Results: As expected, BP was significantly related to age, gender, and body mass index (all $P < 0.001$). Systolic and diastolic BP both decreased significantly with time (all $P < 0.001$). The decrease in systolic and diastolic BP with time was significantly related to age and the maximum cuff pressure (all $P < 0.001$), but was not related to body weight, arm dimension, triceps skinfold thickness, cuff size, and consumption of food, coffee and tobacco in the preceding 30 minutes. For systolic BP, the decrease was also negatively related to the pulse rate ($P < 0.001$).

Conclusion: Older persons and those with high systolic BP had a larger fall in BP on repeated measurements. Therefore, adequate inflation of cuff and repeated measurements in the elderly are key factors for accurate measurement of clinic BP.

Clinical correlates and prognostic implications of cerebral microbleeds in patients with ischaemic stroke

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Introduction: Cerebral microbleeds detected using gradient echo from cerebral magnetic resonance imaging (MRI) has been shown to correlate with various cardiovascular risk factors as well as adverse clinical outcome following ischaemic stroke (ISS). Recently, susceptibility-weighted imaging (SWI) has been noted to be a much more sensitive technique in the detection of cerebral microbleeds. However, literature on clinical correlates and prognostic implications of cerebral microbleeds detected using SWI is scarce.

Methods: We retrospectively reviewed the cerebral MRIs of patients with ISS who were examined at the HKU MRI Unit during 1 March 2008 to 31 December 2012. The location and grading of cerebral microbleeds were scored according to the Microbleed Anatomical Rating Scale. Clinical characteristics, cardiovascular risk factors, medications on discharge after index hospitalisation of ISS, as well as subsequent clinical outcome of study patients were retrieved from the territory-wide clinical management system.

Results: A total of 724 patients with ISS were included in the final analysis; 317 patients were noted to have cerebral microbleeds on cerebral MRI, of which 249, 42, and 26 patients were classified as having microbleeds of grade 1, 2, and 3, respectively. Grading of cerebral microbleeds was significantly correlated with age, underlying hypertension, and glomerular filtration rate (all $P < 0.05$). After adjustment for confounding risk factors, severity of cerebral microbleeds was identified as an independent predictor of subsequent risk of recurrent ISS (hazards ratio [HR]=2.80; 95% confidence interval [CI], 1.06-7.38; $P < 0.05$), and haemorrhagic stroke (HR=3.11; 95% CI, 1.19-8.11; $P < 0.05$). Presence of cerebral microbleeds did not predict other major adverse cardiovascular events nor mortality after ISS.

Conclusions: Cerebral microbleeds detected by SWI from cerebral MRI are strongly associated with age, hypertensive status and renal function, and is an independent predictor of recurrent ISS and haemorrhagic stroke in patients with ISS.

TGF- β 1 induces epithelial-to-mesenchymal transition and increases fibrogenesis in mesothelial cells through decreased expression of miR-200c

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Introduction: Progressive peritoneal fibrosis limits the effectiveness of peritoneal dialysis as a long-term renal replacement therapy in patients with end-stage renal disease. Emerging evidence shows that epithelial-mesenchymal transition (EMT) plays an important role in peritoneal fibrosis but the pathophysiologic mechanisms remain obscure. The aim of this study was to investigate the role of microRNA in peritoneal mesothelial cell EMT.

Methods: The expression profile of different microRNAs was first determined, and miR-200c (which is expressed in the peritoneum) was chosen for further studies focusing on its localisation, functional effect, and downstream processes.

Results: Transforming growth factor beta-1 (TGF- β 1) is an established key mediator in peritoneal fibrosis. Human mesothelial cells exposed to TGF- β 1 showed reduced level of miR-29a, miR-192, miR-200b, miR-200c, miR-324, and miR-377. Corroborating results were obtained in mice given continuous intraperitoneal injection of peritoneal dialysate for up to 5 days. In Met5A mesothelial cell line and peritoneal mesothelial cells isolated from spent dialysate of long-term dialysis patients, over-expression of miR-200c reduced the increase in expression of mesenchymal markers laminin, fibronectin, snail, and fibroblast-specific protein 1 induced by TGF- β 1.

Conclusion: Our results demonstrate the role of microRNAs in EMT of peritoneal fibrosis, and suggest a potential therapeutic role for miR-200c.

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Introduction: Regular exercise habit is essential in maintaining cardiorespiratory fitness (CRF). This study aimed to investigate whether patients with no regular exercise habits prior to entry into a cardiac rehabilitation programme would improve less favourably in CRF after training; and whether more health resource or training would be needed for this group of patients when compared with those having regular exercise beforehand.

Methods: This was a retrospective study. Male subjects were recruited from September 2007 to April 2012 in a cardiac rehabilitation centre of a local hospital. Their exercise habits were investigated before and at the end of programme. Only male patients with regular exercise habit at the end of programme were recruited and their pre-programme exercise habits were traced back for categorisation: "Regular exercise group" (R) if they had pre-programme regular exercise habit, and "Non-Regular exercise group" (NR) if they did not. Six-minute-walk-test (6MWT) and stress test (ST) were used to measure the cardiorespiratory improvement of patients. Outcome measures were the changes in 6MWTs and STs before and at the end of programme. Independent t-test was used to compare the outcome measures between the two groups. Alpha level was set at 0.05.

Results: A total of 276 male patients were recruited in the study. There were 192 patients (age, 62.6 ± 10.2 years) in group R and 84 patients (age, 56.5 ± 10.6 years) in group NR. There was no significant difference between the groups in pre-programme 6MWTs and STs. After training, both groups had improvement in CRF. Group R patients improved from 435.5 ± 87.0 m to 474.1 ± 94.1 m (6MWT) and from 7.3 ± 3.11 MET to 9.31 ± 3.17 MET (ST). Group NR patients improved from 454.4 ± 86.4 m to 505.6 ± 95.4 m (6MWT) and from 7.23 ± 3.35 MET to 9.76 ± 3.17 MET (ST). It showed that patients in group NR did not gain less than group R in 6MWTs and STs.

Conclusion: Male patients with no regular exercise habits did not improve less favourably than those with regular exercise habit. Hence, extra health resource or training is deemed not necessary for them.

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Background: Cigarette smoking is the major risk factor for the development of chronic obstructive pulmonary disease. Cigarette smoke (CS) is a rich source of oxidants, and is thought to disrupt the oxidant-antioxidant balance in the lung, thus inducing apoptosis. Epigallocatechin gallate (EGCG), a natural compound found mainly in green tea, has long been considered as an antioxidant. The aim of this study was to explore the role of EGCG on CS-induced oxidative stress and apoptosis in a rat model of passive smoking.

Methods: Thirty-two male Sprague Dawley rats were equally and randomly divided into four treatment groups: control, CS alone, EGCG alone, and combination of CS and EGCG. The control group was exposed to sham air, while the CS group was exposed to 4% CS for 1 hour daily throughout the experimental period. The EGCG and EGCG + CS groups were given EGCG (50 mg/kg; oral gavage) every other day. Rats were sacrificed 56 days later and protein was extracted from lung tissue. Protein expressions of the antioxidant enzyme quinone oxidoreductase 1 (NQO1), and apoptosis-related protein caspase-3 (total and cleaved) and Bcl-2 were detected by Western blot analysis.

Results: CS exposure alone caused an increase in protein expression of NQO1, Bcl-2, and cleaved caspase-3 in comparison to control group. On the other hand, EGCG alone also increased NQO1 but has no effect on Bcl-2 or caspase-3 (total and cleaved). The combination of EGCG and CS normalised the levels of NQO1 and Bcl-2 protein expression.

Conclusion: Our data reinforce the defensive role of EGCG against oxidative stress and apoptosis. The protective role of EGCG in reversing apoptosis in the CS-exposed rat lungs is thought to be mediated through induction of antioxidant mechanisms.

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Post-ischaemic treatment with melatonin and calpeptin exerts neuroprotective effects against ischaemia/reperfusion injury in a rat model of focal cerebral ischaemia

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Introduction: Melatonin is a potent antioxidant. Previously, we have demonstrated beneficial effects of pretreatment with melatonin in rodent models of focal cerebral ischaemia. Cerebral ischaemia increases intracellular concentration of calcium ion and activates several calcium-dependent proteases such as calpain. Calpeptin is a novel calpain inhibitor. The aim of this study was to investigate the neuroprotective role of post-ischaemia treatment with melatonin and/or calpeptin in transient focal cerebral ischaemia.

Methods: Male Sprague Dawley rats underwent right-sided endovascular middle cerebral artery occlusion (MCAO) for 90 minutes following by 24 hours of reperfusion. An intracerebroventricular injection was initiated 10 to 15 minutes after the onset of reperfusion. Neurological behaviour was assessed using Neurological Deficit Scoring System (NDSS) test, and cerebral infarction volumes were evaluated using tetrazolium staining.

Results: Treatment with either melatonin or calpeptin reduced infarction volume and NDSS score in a dose-dependent manner. Nevertheless, only the high-dose calpeptin group (50 µg/kg) improved both infarction volume ($P=0.046$) and NDSS score ($P=0.001$); the combination treatments of the medium-dose calpeptin (15 µg/kg) and low-dose melatonin (50 µg/kg) exerted synergistic effects.

Conclusion: Our results suggest that post-ischaemia treatment with melatonin and calpeptin via intracerebroventricular route exerts neuroprotective effects against transient focal cerebral ischaemia.

Cognitive function in systemic lupus erythematosus patients with a history of neuropsychiatric manifestations: a longitudinal study

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Background: Cognitive impairment is commonly reported in patients with systemic lupus erythematosus (SLE) and its associations with neuropsychiatric involvement (NPSLE) and psychiatric factors have been inconsistently reported in the literature.

Objective: To evaluate full neurocognitive function in relation to psychiatric factors including anxiety and depression in NPSLE patients longitudinally compared to matched controls.

Methods: Full neurocognitive battery was performed by trained psychologist at two time-points 12 months apart. Depressive and anxiety symptoms were measured by Hospital Anxiety and Depression Scale (HADS).

Results: A total of 18 NPSLE and 18 non-NPSLE patients matched to age, sex, and disease duration as well as 16 age- and sex-matched healthy subjects were recruited. NPSLE patients consistently reported more cognitive impairment and anxiety symptoms than non-NPSLE patients (both $P=0.02$) over both time-points. NPSLE patients had worse performance on three memory tests whereas non-NPSLE patients only showed significantly lower score for Auditory-Verbal Learning Test recognition compared with healthy subjects by post-hoc analysis. Applying age- and education-adjusted Chinese norms, NPSLE patients had significantly worse performance than non-NPSLE patients over five cognitive domains including simple and complex attention, memory, reasoning, and visuospatial function which remained significant when adjusted for HADS-A.

Conclusions: Compared to non-NPSLE patients, NPSLE patients reported more cognitive and anxiety symptoms and had significantly worse cognitive functions involving simple and complex attention, memory, reasoning, and visuospatial domains. Unlike non-NPSLE patients, they failed to demonstrate learning effect upon re-evaluation over 12 months.

Exercise improves glucose homeostasis via enhanced fibroblast growth factor 21 action on adipose tissues

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Introduction: Fibroblast growth factor 21 (FGF21) is a hormone mainly derived from liver and acts on adipocytes by activating FGF21 receptor (FGFR)-mediated intracellular signalling. FGF21 can inhibit lipolysis and stimulate adiponectin production in adipose tissues. Several lines of evidence shows that exercise can regulate FGF21 action on target tissues by affecting FGF21 expression and sensitivity. This study aimed to investigate whether the beneficial effects of exercise on glucose homeostasis are mediated by enhanced FGF21 action on adipose tissues and clarify involving molecular mechanisms.

Methods: Both wild type (WT) and FGF21 knockout (KO) mice were fed with high-fat diet (HFD) for 2 months and then divided into sedentary and exercised groups. Exercised mice were subjected to treadmill running for another 1 month. Body composition and glucose tolerance (GT) were monitored. Blood and tissue samples were collected for biochemical, histological, and molecular analysis.

Results: Chronic exercise could increase expression of FGFR in adipose tissues in HFD-induced obese mice. KO mice had significantly higher serum free fatty acids (FFA) level ($P<0.01$) and lower adiponectin level ($P<0.05$) than WT mice after exercise. Exercise slightly decreased fat mass (11.98%, $P=0.24$) and fat percentage (6.23%, $P=0.45$) of WT mice, but significantly reduced fat mass (34.24%, $P=0.001$) and fat percentage (24.22%, $P=0.001$) of KO mice. However, exercise could significantly improve GT of WT mice ($P<0.001$), but not KO mice ($P=0.25$). Besides, exercise could significantly reduce liver triglyceride and inhibit activation of JNK in WT mice, but not KO mice.

Conclusions: Chronic exercise can increase FGFR expression and enhance FGF21 action on adipose tissues. Enhanced FGF21 action on adipose tissues can inhibit exercise-induced excessive lipolysis and promote adiponectin production. Without FGF21, exercise-induced excessive FFA will be accumulated, which subsequently induces triglyceride deposition and JNK activation in liver, leading to systemic glucose intolerance.

Adiponectin promotes macrophage proliferation

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Introduction: Beige cell, the brown-like adipocytes scattered in white adipose tissue, has recently been identified in both human and mammals. Recent studies have demonstrated that cold-induced adipose tissue browning was largely dependent on the local secretion of catecholamine from M2 macrophages. Adiponectin has been reported to promote macrophage polarisation to M2 stage. But whether it is involved in M2 macrophage biology during adipose tissue browning is unclear.

Methods: Wild type (WT) and adiponectin knockout mice (KO) were housed at cold temperature to induce adipose tissue browning. The presence of macrophages in adipose tissue was measured by flow cytometry, quantitative polymerase chain reaction, and immunostaining. EdU incorporation assay and expression of Ki67 were used to evaluate the macrophage proliferation both in vitro and in vivo. Macrophage was differentiated in vitro from bone marrow.

Results: Cold-induced macrophage accumulation was greatly impaired in adiponectin KO mice. In-vitro studies demonstrated that adiponectin could potently enhance M2 macrophage proliferation. Cold challenge led to an increase of T cadherin, the binding coreceptor of adiponectin. Meanwhile, the expression of T cadherin was significantly elevated in M2 macrophages compared to the M1 subtype, while the expression of adiponectin receptor 1 and 2 remained unaltered. Knocking down of T cadherin in bone marrow-derived macrophages caused dramatic decrease of adiponectin binding. More importantly, knocking down of T cadherin led to impairment of adiponectin-evoked macrophage proliferation and Akt phosphorylation.

Conclusion: Adiponectin promotes M2 macrophage proliferation. This is mediated by its coreceptor T cadherin and the downstream target Akt.

Endothelin-1 overexpression exacerbates experimental autoimmune encephalomyelitis

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Multiple sclerosis (MS) is a central nervous system inflammatory demyelinating disorder. T helper 1 (Th1) and T helper 17 (Th17) cells are important in MS immunopathogenesis. Level of endothelin-1 (ET-1), a vasoconstrictor, is increased in sera of MS patients. We studied the role of ET-1 in experimental allergic encephalomyelitis (EAE), a MS animal model. EAE was induced in transgenic mice overexpressing endothelial ET-1 (TET-1), transgenic mice overexpressing astrocytic ET-1 (GET-1) and non-transgenic (NTg) mice by immunisation with myelin oligodendrocyte glycoprotein (MOG)₃₅₋₅₅ peptide. EAE scores, spinal cord histology, serum proinflammatory cytokines levels, and proinflammatory cytokines production from splenocytes of ET-1 transgenic and NTg mice with EAE were studied. ET-1 transgenic mice developed more severe EAE than NTg with increased inflammation and demyelination in spinal cord. The mean maximum EAE scores for GET-1, TET-1 and NTg mice with EAE were 4.84, 4.31 and 4.05 respectively ($P < 0.05$). Serum levels of interleukin (IL)-6, IL-17A, interferon (IFN)- γ and tumour necrosis factor (TNF)- α were higher in ET-1 transgenic than NTg mice with EAE ($P < 0.05$) while serum IL-4 levels were similar. mRNA levels of IL-6, IL-17A, IFN- γ and TNF- α from cultured splenocytes were higher in ET-1-transgenic than NTg mice with EAE ($P < 0.05$), while IL-4 mRNA levels were similar. Consistently, levels of IL-6, IL-17A, IFN- γ and TNF- α in culture media of splenocytes were higher in ET-1 transgenic than NTg mice with EAE ($P < 0.05$), while IL-4 levels were similar.

We concluded that mice with endothelial or astrocytic ET-1 overexpression developed more severe EAE with increased splenic lymphocytes production of Th1 and Th17 proinflammatory cytokines.

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

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

Methods: Gene expression of *arl4aa* and other haematopoietic genes were evaluated by both whole-mount in-situ hybridisation (ISH) and quantitative reverse-transcription polymerase chain reaction (PCR). Knock-down (KD) and knock-out of *arl4aa* was achieved by morpholinos and transcription activator-like effector nuclease (TALEN), respectively. Comparison of data was evaluated by Students' unpaired *t*-test.

Results: *arl4aa* was expressed in the intermediate cell mass at 18hpf, along the dorsal aorta (DA) at 36hpf and the caudal haematopoietic tissue (CHT) at 48hpf. KD of *arl4aa* resulted in reduction of *c-myb* expression (a marker of definitive haematopoietic stem cells [HSC]) in the DA and CHT at 36 and 48hpf and *rag1* expression in the thymus at 96hpf (a marker of T-lymphoid development that arise from definitive HSC). Primitive macrophage development was increased, as shown by increased *l-plastin* and *mpeg1* expression over the yolk sac at 24 hpf. Two TALEN pairs targeting Exon1 and Exon2 of *arl4aa* were designed. Injection of either TALEN pair of Exon1 or Exon2 introduced small insertions and deletions at targeting site as shown by PCR-RFLP assay. Co-injection of both TALEN pairs resulted in a large deletion of ~5kb genomic fragments. *C-myb* expression was analysed in F0 embryos in which large fragments were deleted as detected by single embryo genotyping. ISH result showed *c-myb* expression disappeared in 22.2% F0 embryos and decreased in 50% F0 embryos.

Conclusion: We demonstrated an undescribed function of *arl4aa* during embryonic haematopoiesis in regulating both primitive macrophage and definitive HSC development. The underlying mechanisms are being investigated.

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Functions of *flt3* in zebrafish haematopoiesis and its relevance to human acute myeloid leukaemia

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Introduction: FMS-like tyrosine kinase 3 (FLT3) is expressed in human haematopoietic stem and progenitor cells (HSPCs) but its role during embryogenesis is unclear. Internal tandem duplication (ITD) and tyrosine kinase domain (TKD) mutations of FLT3 were reported in 30% of acute myeloid leukaemia (AML) patients and are associated with inferior clinical prognosis.

Methods: We made use of zebrafish to examine the role of *flt3* in developmental haematopoiesis and model human FLT3-ITD⁺ and FLT3-TKD⁺ AML by injecting DNA or mRNA into the zebrafish embryos.

Results: Zebrafish *flt3* were remarkably similar to their mammalian orthologs. *flt3* knockdown by morpholino significantly reduced the expression of *l-plastin* (pan-leukocyte), *csf1r*, and *mpeg1* (macrophage), *c-myb* (definitive HSPCs), *lck*, and *rag1* (T-lymphocyte). Expressing human FLT3-ITD by plasmid DNA in zebrafish embryos resulted in expansion and clustering of myeloid cells (*pu.1*⁺, *mpo*⁺, and *cebpa*⁺), which were ameliorated by AC220 and associated with *stat5*, *erk1/2*, and akt phosphorylation. Human FLT3-TKD (D835Y) induced significant, albeit modest, myeloid expansion resistant to AC220. Intriguingly, expressing human FLT3-ITD by mRNA injection in zebrafish embryos significantly upregulated follistatin (*fst*) and resulted in a dorsalisation and axis duplication phenotype. As an antagonist of transforming growth factor- β family members, *FST* was significantly increased in FLT3-ITD⁺ leukaemia, which provided a novel mechanism underlying the proliferation advantage of FLT3-ITD⁺ leukaemia cells.

Conclusion: This study provides novel insight into the role of *flt3* during haematopoiesis and establishes a zebrafish model of FLT3-ITD⁺ and FLT3-TKD⁺ AML that may facilitate high-throughput screening of novel and personalised agents.

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Elevated level of neurotoxic α -synuclein oligomers in cerebrospinal fluid of aged Parkinsonian leucine-rich repeat kinase 2 (LRRK2) R1441G mutant mice

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Introduction: Parkinson's disease (PD) is characterised by selective loss of dopaminergic neurons in the brain substantia nigra par compacta. Brain accumulation of misfolded α -synuclein aggregates in Lewy bodies is one of the major hallmarks of PD. Identification of neurotoxic oligomeric α -synuclein in body fluid is considered a biomarker of PD. Leucine-rich repeat kinase 2 (LRRK2) mutation is the commonest genetic risk of PD with age-related penetrance, but the pathogenic mechanism is unclear. Alpha-synuclein is physiologically metabolised and degraded via chaperone-mediated autophagy (CMA) in neurons. Evidence showed that LRRK2 mutation may impair CMA, which may subsequently affect α -synuclein level in neurons and the cerebrospinal fluid (CSF). Here we studied the pathogenic consequence of LRRK2^{R1441G} mutation in the levels of α -synuclein and its oligomers in brain CSF of young and aged mutant mice.

Methods: Mouse CSF was extracted by cisterna magna puncture using glass capillary tubes. Purity of CSF was assessed by spectrophotometric scan (200-750 nm) for blood contamination. The levels of total and oligomeric α -synuclein in the CSF of young (3 months old) and aged (18-24 months old) LRRK2^{R1441G} knockin mice and their wildtype littermates were assessed by dot blotting and enzyme-linked immunosorbent assay.

Results: Levels of α -synuclein oligomers were significantly elevated in the CSF of aged, but not young LRRK2^{R1441G} mutant mice when compared with their age-matched wild-type littermates.

Conclusion: Elevation of α -synuclein oligomers in CSF of aged LRRK2^{R1441G} knockin mice is consistent with the increased level of CSF oligomers in human asymptomatic LRRK2 mutation carriers. Our findings indicate that LRRK2 mutation may potentiate formation and release of toxic α -synuclein oligomers into CSF, which may be a useful early biomarker for LRRK2-associated PD.

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Environmental estrogenic endocrine disruptive compounds suppress catechol-O-methyltransferase expression in vitro and in vivo: implication of environmental impacts on neurological disorders

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Introduction: Endocrine disrupting chemicals (EDCs) of trace amounts can interfere with hormonal system in humans. 4-nonylphenol (4-NP) and bisphenol-A (BPA) are two major estrogenic contaminants in Hong Kong regional seawater, sediments, and sewage effluents. How these environmental pollutants affect central nervous system is unclear. Human catechol-O-methyltransferase (COMT) maintains homeostasis of catecholamines (eg dopamine) in the brain, and is physiologically regulated by estrogen-mediated gene transcription mechanisms. We aimed to study if exposure to environmental estrogenic EDCs can modulate COMT expression in neurons.

Methods: We measured changes of COMT protein expression in (1) mouse primary cortical neurons after treatment of either 4-NP (450 nM), BPA (450 nM), or sea sediment extracts containing these EDCs, and (2) in C57BL/6 mouse brain dopaminergic regions (ie frontal cortex and striatum) 2 days after direct intracerebroventricular injection of EDCs (4-NP or BPA [100 nM in 3 µL aCSF per mouse]).

Results: Incubation of 4-NP, BPA, or sea sediment extracts in primary cortical neurons (DIV9) for 72 hours caused marked reduction in COMT expression, which can be antagonised by ICI182780 (estrogen-receptor antagonist). Mouse brain injected with either 4-NP or BPA for 2 days also significantly reduced COMT expression in soluble lysates extracted from frontal cortex and striatum.

Conclusion: Our results demonstrated that 4-NP and BPA suppress COMT expression via classical estrogen receptor pathway, and further affirmed the use of COMT assay to assess endocrine disruptive potential of various environmental samples (granted patents in US, Europe and China [US20100081147A1; EP2328909; CN102203119A]). Our findings will benefit elucidation of yet underestimated pathogenic events from EDCs absorption, and suggested that environmental EDCs exposure may perturb dopamine balance via COMT in various neurophysiological dysfunctions.

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Bringing an old drug to a new treatment strategy in treating FLT3-ITD⁺ AML-combination of homoharringtonine (HHT) and sorafenib

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Introduction: A gain-of-function internal tandem duplication (ITD) of fms-like tyrosine kinase 3 (*FLT3*) was found in 30% of acute myeloid leukaemia (AML) and was associated with an inferior treatment response and clinical prognosis. Despite much interests in FLT3 inhibitors in clinical trials, the response was at best transient, limiting their clinical application. Homoharringtonine (HHT) is a protein translation inhibitor and affects primarily proteins with short half-lives, including many of the downstream effectors of FLT3 signalling. In this study, we evaluated if HHT can be used in combination with sorafenib in the treatment of FLT3-ITD⁺ AML and examined the mechanistic basis of their synergism.

Methods: The anti-leukaemia effects of drugs on AML cell lines with or without FLT3-ITD mutation were evaluated by Prestoblue assay. The drug effects on leukaemia-initiating cells activity were examined by xenotransplantation using NSG mice and percentage of human cells engraftment were examined after 6 weeks. The effect of HHT and FLT3 inhibitors on FLT3 signalling were examined by Western blot.

Results: HHT exhibited more potent growth inhibitory effect on FLT3-ITD⁺ AML cell lines, MV4-11, and MOLM-13 (IC₅₀: 3.65 and 3.67 nM) than other AML cell lines (IC₅₀: 7.7-32.3 nM). Combination of HHT and sorafenib (H+S) showed pronounced synergism in growth inhibition based on EOBA (29.7%±5%). H+S in vitro significantly reduced engraftment of MV4-11 cells (Vehicle: 65.0%±9.7%; H+S: 21.8%±8.7%, P<0.01, n=6) in NSG mice. Synergism between H+S was also seen in primary AML samples. Mechanistically, HHT treatment for 6 hours reduced total FLT3 and p-FLT3 protein levels in MV4-11. Protein levels of downstream effectors of FLT3 pathway including total Stat5, pStat5, pStat3, pErk were also reduced. A phase II clinical trial of sorafenib and HHT combination treatment in patients with chemo-refractory FLT3-ITD⁺ AML has begun in Hong Kong since January 2014. Five patients have been treated, including two patients who were primarily refractory to sorafenib monotherapy. Complete remission (CR) was achieved in one patient and CR with incomplete haematological recovery (CRi) in four others after 1 cycle. The FLT3-ITD allelic burdens before treatment and at CR/CRi were 77.7±9.6% and 20.0±9.6% (P=0.007).

Conclusions: HHT and sorafenib demonstrated significant synergistic effect in suppressing the growth of FLT3-ITD⁺ AML cells both in vitro and in vivo. It provides a promising strategy in improving treatment outcome of FLT3-ITD⁺ AML patients.

Left ventricular diastolic function changes in end-stage renal disease patients with peritoneal dialysis

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Introduction: Peritoneal dialysis (PD) is the major therapy for the end-stage renal disease (ESRD) patients. Cardiovascular disease remains the leading cause of mortality and morbidity in these patients, and left ventricular (LV) diastolic dysfunction has a high prevalence. We aimed to evaluate LV diastolic function changes and to determine the association of LV diastolic function and peritoneal solute clearance of the ESRD patients undergoing PD.

Methods: We recruited 82 patients who were treated with maintenance home PD therapy and who were observed for 1 year. We assessed the baseline LV diastolic function, residual renal function, dialysis adequacy and peritoneal equilibration test (PET), and measured again 1 year later.

Results: There were increased ratio of peak early diastolic transmitral inflow velocity to average peak early diastolic mitral annular velocity (avg.E/E'), the ratio of peak systolic velocity to peak early diastolic velocity of pulmonary venous inflow (S/D), pulmonary artery diastolic pressure, minimum left atrial volume index (LAVI_{min}), left atrial volume index at onset of p wave (LAVI_p), dialysate-to-plasma ratio of creatinine (D/Pcr), and decreased D, left atrial active empty rate (LAVI_{act}%), left atrial ejection fraction, end-to-initial dialysate glucose (D/Do) at 1 year. The study showed that there were strong relationships between left ventricular mass index (LVMI), D/Pcr, D/Do and avg.E/E', and LVMI was an independent predictor of avg. E/E' in ESRD patients undergoing PD.

Conclusion: LV diastolic function had a decreased trend in the PD patients, and D/Pcr, D/Do and LVMI were strongly associated with avg.E/E'. In addition, LVMI was an independent predictor of avg.E/E' in ESRD patients undergoing PD.

The role of a novel G protein-coupled receptor GPR110 in the pathogenesis of non-alcoholic fatty liver diseases

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Introduction: The liver plays a crucial role in regulating metabolic homeostasis, especially in mobilising fatty acids. GPR110 is strongly down-regulated in the liver of obese mice. Although we found mice lacking GPR110 have obese phenotype and slightly higher serum free fatty acid (FFA) level, they are metabolically healthier than their wild-type littermates under a high-fat diet feeding. This study aimed to investigate the mechanism underlying this phenotype.

Methods: The adenovirus gene delivery system was developed to over-express GPR110 in mouse liver, and GPR110 knockout (KO) mice were generated. Glucose and insulin tolerance tests were performed. Serum lipid profiles were checked. Mice were sacrificed and liver tissues were collected for assessments of hepatic lipid accumulation by Oil-Red O staining. Yeast two-hybrid assay was performed to identify the binding partner of GPR110.

Results: Over-expression of GPR110 in the liver of obese mice increased hepatic lipid accumulation and impaired glucose homeostasis and insulin sensitivity. Whilst, GPR110 KO mice showed reduced lipid contents in the liver, and delayed progression to diet-induced glucose intolerance. Yeast two-hybrid assay identified IQGAP2, an important protein that regulates FFA uptake by CD36, as the binding protein of GPR110. More importantly, IQGAP2 KO mice were reported to have similar phenotypes as that of our GPR110 KO mice.

Conclusion: This study demonstrates a significant role of GPR110 in regulating lipid metabolism in the liver via IQGAP2 and CD36. The decline of GPR110 in the liver of obese mice may play a protective role in the pathogenesis of non-alcoholic fatty liver diseases.

Adiponectin promotes adipose tissue browning by enhancing macrophage proliferation

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Introduction: Beige cell, the brown-like adipocytes scattered in white adipose tissue, has recently been identified in both human and mammals. Ways to enhance beige cell are demonstrated to antagonise obesity and its related diseases. Adiponectin possesses versatile functions against a cluster of metabolic disorders, but the exact function of this adipokine in beige cell biogenesis is unknown.

Methods: Wild type (WT) and adiponectin knockout (KO) mice were housed at cold temperature and the adipose browning was evaluated by Western blotting, immunohistochemistry and/or quantitative polymerase chain reaction analysis of UCP-1 expression. The presence of macrophages in adipose tissue was measured by flow cytometry and immunostaining. EdU incorporation assay was performed to determine the macrophage proliferation.

Results: Cold challenge led to remarkable accumulation of adiponectin in inguinal adipose tissue which was accompanied by enrichment of M2 macrophages. Depletion of macrophages in adipose tissue largely abolished cold-induced adipose tissue browning. Cold-induced browning and macrophage accumulation was greatly impaired in adiponectin KO mice. EdU imaging demonstrated that the increased M2 macrophage was not derived from circulating monocytes, but from de-novo macrophage proliferation.

Conclusion: Adiponectin plays a pivotal role in controlling beige cell biogenesis. Adiponectin achieves its pro-browning effects by both direct activity on adipocytes and indirectly through promoting local proliferation of M2 macrophages.

Risk factors and post-resection independent predictive score for the recurrence of hepatitis B-related hepatocellular carcinoma

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Background: Independent risk factors associated with hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC) after resection remains unknown. An accurate risk score for HCC recurrence is lacking.

Methods: We prospectively followed up 200 patients who underwent liver resection for HBV-related HCC for at least 2 years. Demographic, biochemical, tumour, virological, and anti-viral treatment factors were analysed to identify independent risk factors associated with recurrence after resection and a risk score for HCC recurrence formulated.

Results: Two hundred patients (80% male) who underwent liver resection for HBV-related HCC were recruited. One hundred patients developed HCC recurrence (median duration after resection, 52 weeks). Multivariate analysis identified that the presence of lymphovascular permeation ($P < 0.001$; relative risk [RR]=2.63), microsatellite lesions ($P < 0.001$; RR=2.56), preoperative HBV DNA of $> 20\,000$ IU/mL ($P = 0.028$; RR=1.62) were independently associated with HCC recurrence. Antiviral treatment before ($P = 0.008$; RR=0.07) and after ($P = 0.004$; RR=0.55) resection was independently associated with lower risk of HCC recurrence. A post-resection independent predictive score (PRIPS) was derived and validated with sensitivity of 72.1% and 69.8% and specificity of 62.9% and 77%, to predict the 1- and 3-year risks for the HCC recurrence respectively with the hazard ratio of 2.71 ($P < 0.001$). The area under the curve for the 1- and 3-year prediction were 0.69 and 0.78, respectively.

Conclusions: Several tumour and virological factors were associated with a higher cumulative risk of HCC recurrence after resection. PRIPS was derived for more accurate risk assessment. Antiviral treatment reduced the risk of recurrence.

Immunogenicity of intradermal trivalent influenza vaccine with topical imiquimod: a double-blind randomised controlled trial

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Background: Imiquimod, a synthetic toll-like receptor 7-agonist enhanced immunogenicity of influenza vaccine in a mouse model. We hypothesised that topical imiquimod before intradermal trivalent influenza vaccination (TIV) will produce similar effect in human.

Methods: We performed a prospective 1-year follow-up double-blind randomised controlled trial on adults with co-morbidities. Subjects were randomised to one of the three vaccinations: topical 5% 250 mg imiquimod ointment followed by intradermal TIV (Intanza[®]15, Sanofi-Pasteur, France), or topical aqueous-cream followed by intradermal TIV, or topical aqueous-cream followed by intramuscular TIV (Vaxigrip[®], Sanofi-Pasteur, France). Patients and investigators were blinded to the type of topical treatment applied. Haemagglutination inhibition (HI) and microneutralisation antibody titres were measured. Primary outcome was day-7 seroconversion rate.

Results: A total of 91 recruited subjects completed the study. The median age was 73 years. On day 7, 27/30 (90%) patients who received imiquimod and intradermal TIV achieved seroconversion against the H1N1 strain by HI, compared to 4/30 (13.3%) who received aqueous-cream and intramuscular TIV ($P < 0.001$) and 12/31 (38.7%) who received aqueous-cream and intradermal TIV ($P < 0.001$). The seroconversion, seroprotection, and geometric mean titre fold increase were met in all three strains in the imiquimod and intradermal TIV group 2 weeks earlier, and the better seroconversion rate was sustained from day 7 to year 1 ($P \leq 0.001$). The better immunogenicity was associated with less hospitalisation for influenza or pneumonia ($P < 0.05$). All adverse reactions were self-limited.

Conclusions: Pretreatment with topical imiquimod significantly expedited, augmented, and prolonged the immunogenicity of influenza vaccination. This strategy for influenza immunisation should be considered in the elderly population.

Note

The study was published in the *Clinical Infectious Diseases*: Hung IF, Zhang AJ, To KK, et al. Immunogenicity of intradermal trivalent influenza vaccine with topical imiquimod: a double blind randomized controlled trial. *Clin Infect Dis* 2014;59:1246-55.

Absolute benefits and harm of dual antiplatelet therapy after drug eluting stenting

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Background: The optimal duration of dual antiplatelet therapy (DAPT) following recourse to drug-eluting stenting for coronary heart disease has generated controversy. The findings of the recently published DAPT trial involving 9961 randomised patients shed some light on the possible benefits and harm of extending such treatment from 12 to 30 months expressed in relative terms.¹ However, understanding the findings of this double-blind, multicentre clinical trial comprehensively requires that absolute benefits and harms should also be considered.

Methods: As previously described,² we derived unadjusted estimates of relative risk (RR) and number needed to treat (NNT)/year values for extended DAPT (clopidogrel or prasugrel + aspirin) versus aspirin only, based on the published DAPT trial results.

Results:

No. of randomised patients	Pre-specified endpoint	% RR (95% CI)	NNT/year* (95% CI)
DAPT: 5020	Stent thrombosis†	29 (17 to 48)	160 (115 to 263)
Aspirin + placebo: 4941	MACCE‡	73 (61 to 87)	96 (62 to 217)
(Follow-up: 1.5 years)	Death from any cause	130 (96 to 177)	-330 (-154 to 2240)
	Severe or moderate bleed	160 (119 to 216)	-168 (-437 to -104)

* Negative NNT values indicate harm

† Definite or probable

‡ Major adverse cardiovascular or cerebrovascular event (death, myocardial infarct, stroke)

Discussion & Conclusion: These unadjusted parameters for extended DAPT duration reveal clearly significant relative and absolute benefits with respect to stent thrombosis and major cardiovascular and cerebrovascular events and harm with respect to severe or moderate bleeds, but with respect to deaths the absolute harm was small and not quite statistically significant.

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Comprehensive Index of Frailty: a multi-dimensional construct from the Hong Kong Centenarian Study

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Introduction: Frailty is a global epidemiological and clinical phenomenon that can lead to poor long-term outcome. A better understanding of its components is essential for future developments of management strategies. We sought to assess the incremental validity of a new Comprehensive Index of Frailty over Frailty Index in predicting self-rated health and functional dependency among the oldest-old adults.

Methods: We conducted a cross-sectional community-based centenarian study. A quota sampling method was used to recruit a geographically representative sample of 124 community-dwelling Chinese near- and centenarians. Two validated instruments (Chinese Longitudinal Healthy Longevity Survey and Elderly Health Centre questionnaire) were administered through face-to-face interviews. Frailty was first assessed using a 32-item Frailty Index (FI-32). Then a new Comprehensive Index of Frailty (CIF) was constructed by adding 12 more items in the psychological, social/family, environmental, and economic domains to the FI-32. Hierarchical multiple regression was used to explore whether the new CIF provided significant additional predictive power for self-rated health and instrumental activities of daily living (IADL) dependency.

Results: The mean age was 97.7 (standard deviation, 2.3; range, 95-108) years, and 74.2% were female. Using the Frailty Index for reference, 16% of our participants were non-frail, 59% were pre-frail, and 25% were frail. Frailty according to FI-32 significantly predicted self-rated health and IADL dependency beyond the effect of age and gender. Inclusion of the new CIF into the regression models provided significant additional predictive power beyond FI-32 on self-rated health, but not IADL dependency.

Conclusions: Psychological, social/family, environmental, and economic factors are essential elements of a frailty assessment tool. Our result supports the concept that a comprehensive model of frailty should be a multi-dimensional and multi-disciplinary construct. Future studies should validate this construct in different settings and age-groups, using our new CIF.

Adipose-specific inactivation of c-Jun NH2-terminal kinase alleviates atherosclerosis in ApoE-deficient mice

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Introduction: Inflammation in adipose tissues is observed in obesity, a major risk factor for atherosclerosis. This study aimed to investigate whether adipose-specific inactivation of c-Jun NH₂-terminal kinase (JNK) can protect against atherosclerosis.

Methods: Transgenic mice expressing an adipose-specific dominant negative form of JNK (dnJNK) were crossbred with ApoE^{-/-} mice to generate ApoE^{-/-}/dnJNK (ADJ) mice. ApoE^{-/-} and ADJ mice were fed a high-fat-high-cholesterol diet for 10 weeks and examined for atherosclerosis, adipose tissue inflammation, and metabolic phenotypes. For transplantation study, epididymal white adipose tissues (eWAT) from dnJNK or wild-type C57 donors were transplanted into ApoE^{-/-} recipients, which were subjected to atherosclerosis assessment.

Results: ADJ mice developed significantly less atherosclerotic plaques in the aorta and aortic root, as shown by Oil Red O staining. Macrophage infiltration and the expression of pro-inflammatory cytokines in adipose tissues were markedly reduced in eWAT in ADJ mice. ApoE^{-/-} mice receiving eWAT transplantation from ADJ donor mice, but not wild-type donor mice, were protected from atherosclerosis, as shown by Oil Red O staining.

Conclusion: JNK inactivation in adipose tissues can alleviate atherosclerosis, suggesting a therapeutic potential in atherosclerosis management.

Acknowledgement

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Elevation of low-profile and traditional percutaneous endoscopic gastrostomy replacement tube

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Introduction: Extra-abdominal part of percutaneous endoscopic gastrostomy (PEG) tubes is usually bulky. Life span of the placement tubes mainly depends on the water-filled balloon at the tip. Low-profile non-balloon type replacement tubes are developed to overcome these shortcomings. Recently, the safety of these low profile tubes is criticised.

Methods: This was a retrospective case-matched study of PEG clinic patients in Tung Wah Hospital from 1 January 2008 to 30 June 2014. All 17 patients receiving low-profile non-balloon type replacement tubes were recruited as study group. Another 34 patients receiving usual balloon type replacement tubes with matched demographics and causes of dysphagia were recruited as control group. Records were reviewed to identify complications leading to tube removal and life span of the replacement tubes.

Results: Three patients in the study group required tube removal due to buried bumper syndrome but only one patient in control group required tube removal due to exit site infection. There was no statistical significance of the incidence between these two groups ($P=0.066$). The tube life span of the replacement tube in study group was extremely statistically significantly longer than control group (study group 395.20 days vs control group 173.80 days; $P<0.0001$).

Conclusion: The safety of low-profile non-balloon type PEG replacement tube is still comparable with traditional balloon type replacement tube. However the statistical significance is only marginal. Furthermore, the reason for tube removal in study group patients were all due to buried bumper syndrome, which is a severe complication and may be life threatening. Caution about the daily care of the low-profile replacement tube should be emphasised to the patients and caregivers. With good aftercare, the low-profile replacement tube is much more durable, more cost effective, and comfortable.

The prevalence of potentially inappropriate prescribing among Hong Kong older adults: a comparison among Beers 2003, Beers 2012, and STOPP/START criteria

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Introduction/Objectives: Inappropriate prescribing can lead to adverse drug events and older adults are at greater risk due to multiple chronic co-morbidities and multiple medications prescribed. Various criteria (eg Beers or STOPP [Screening Tool of Older Person's Prescriptions]) on medication usage have been developed to assess prescribing appropriateness. The objectives of this study were (1) to determine the prevalence of potentially inappropriate prescribings (PIPs) among Hong Kong older adults using 2003 Beers, 2012 Beers, and STOPP criteria, and (2) to evaluate the association between number of medications and PIPs.

Methods: A retrospective, observational, chart review study was carried out. All three criteria were used to assess PIPs and instances of PIPs were recorded.

Results: A total of 500 patients (246 males and 254 females; mean age \pm standard deviation [SD], 81.45 ± 8.61 years) were recruited. Overall, 3997 medication items (mean number of medications per person \pm SD, 7.99 ± 4.53) were reviewed; 233, 374, and 220 PIPs were identified by 2003 Beers, 2012 Beers, and STOPP criteria, respectively. 2012 Beers criteria identified more PIPs than the other two criteria ($P<0.001$). There was an association between number of medications and PIPs (Spearman's $\rho=0.335, 0.352, 0.384$ respectively; all $P<0.05$).

Conclusion: This is the first local study that examines the prevalence of PIPs using Beers and STOPP criteria. Polypharmacy is common among the older adults. The 2012 Beers criteria appear to identify more PIPs than the other two criteria. This study reinforces that careful prescribing is necessary as more medications can increase PIPs which can lead to increased risk of adverse drug events.

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Massive degradation in FGFR/Akt/Erk signalling by arsenic trioxide and FGFR inhibitor PD173074 in squamous cell lung carcinoma SK-MES-1

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Introduction: Lung cancer is the top cancer killer. Squamous cell carcinoma (SCC) represents the second most common histological subtype of lung cancer. Arsenic trioxide (ATO) has been demonstrated to inhibit tumour growth in lung adenocarcinoma and initiate apoptosis in acute promyelocytic leukaemia. Fibroblast growth factor (FGF) receptor (FGFR) amplification is shown in some SCC. FGFR inhibitor (eg PD173074) has been developed to inhibit FGFR.

Methods: The combination effect of ATO and PD173074 was studied using a cell line with FGFR amplification: SK-MES-1. The effect of ATO and/or PD173074 on cell viability and protein expression was studied by MTT assay and Western blot, respectively. Cell cycle arrest, phosphatidylserine externalisation, and mitochondrial membrane depolarisation were monitored by flow cytometry. FGFR knockdown was performed with siRNA targeting FGFR. Proteasome inhibitor (MG-132) was used to study the degradation mechanism. The in-vivo effect of ATO and/or PD173074 was investigated with a nude mice xenograft model.

Results: Combination of ATO and PD173074 reduced cell viability along with increased sub-G1 population, phosphatidylserine externalisation and mitochondrial membrane depolarisation more significantly than single drug alone. In general, downregulation of FGFR, p-Akt, Akt, p-Src, Src, p-c-Raf, c-Raf, Erk, Bcl-2 and survivin as well as upregulation of p-Erk and cleaved PARP were observed upon ATO and/or PD treatment with or without FGF. MG-132 reversed the degradation of Akt, Src, c-Raf and Erk induced by ATO/PD, but not FGFR, which disclosed proteasome degradation system was involved. Downregulation of FGFR, Akt, Src, c-Raf and Erk as well as cleaved PARP elevation induced by ATO and/or PD were confirmed in vivo.

Conclusion: Massive protein degradation (FGFR, Akt, Src, c-Raf and Erk) was induced by ATO and/or PD173074 treatment mainly mediated by activation of proteasome degradation system in SCC cell line SK-MES-1 in vitro and in vivo.

Development of a clinically relevant in-vitro drug screening platform for chemo-refractory acute myeloid leukaemia patients

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Introduction: The cure rate of acute myeloid leukaemia (AML) has remained disappointing despite advances in the use of chemotherapy and bone marrow transplantation. The heterogeneous nature of AML in terms of cytogenetic and genetic abnormalities, clinicopathological characteristics, and response to therapies are believed to attribute to the failure of the present standard induction and consolidation regimen. We proposed to develop and optimise an in-vitro drug screening platform using primary AML samples with results validation based on translation into xenograft mouse models and clinical correlation.

Methods: Mononuclear fraction enriched in blasts was extracted from either peripheral blood or bone marrow aspirates of AML patients by density-gradient centrifugation, and cultured in vitro on 96-well plates in various culture conditions and exposed to a library of drugs, including tyrosine kinase inhibitors and chemotherapeutic drugs currently used in treating malignancies. A resazurin-based cell viability agent was used 3 days later in a high-throughput scale to measure their cytotoxic effects on a variety of samples.

Results: Among the 100 samples currently screened, the drugs exhibited varying effects on the primary AML samples. Among the molecules which produced variable effects across the patient samples, we initially verified the FLT3-ITD mutation correlated with sensitivity towards FLT3 inhibitors, and are looking into possible correlation to in-vivo drug response in immunocompromised mice engrafted with the particular samples.

Conclusion: This platform provides a drug response profiling of the heterogeneous AML blasts which could help design personalised medicine for patients in clinical settings.

Continuous entecavir for treatment-naïve Chinese chronic hepatitis B patients: the seven-year results

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Introduction: There is paucity of data on uninterrupted entecavir for treatment-naïve chronic hepatitis B (CHB) beyond 6 years.

Methods: Treatment-naïve Chinese CHB patients were treated continuously with entecavir for up to 7 years. The cumulative rates of HBV DNA undetectability, alanine aminotransferase (ALT) normalisation, hepatitis B e-antigen (HBeAg) seroconversion, virological breakthrough, hepatitis B surface antigen (HBsAg) reduction, and genotypic resistance to entecavir were determined. HBV DNA levels were measured by Roche Taqman real time polymerase chain reaction assay. Resistance profile was determined by line probe assay (LiPA, Innogenetics NV, Gent, Belgium). Measurement of serum HBsAg levels was performed using Elecsys HBsAg II assay (Roche Diagnostics, GmbH, Mannheim).

Results: A total of 222 Chinese CHB patients (median age, 45 years; 70.7% male) were recruited. Of them, 222, 188, 173, 170, 167, 162, and 160 patients were followed up for 1, 2, 3, 4, 5, 6, and 7 years, respectively. The cumulative rate of HBV DNA undetectability, ALT normalisation, and HBeAg seroconversion (90 patients were HBeAg positive at baseline) were 98.7%, 98.3%, and 90.2% up to year 7, respectively. The cumulative rate of virologic breakthrough was 8.3%. Entecavir signature mutations were found in two patients. The cumulative rate of entecavir resistance of up to 7 years was 1.2%. The median rate of HBsAg reduction over 6 years of treatment was 0.095 log IU/mL/year. Of the patients, 15.2% and 7.9% patients had HBsAg levels of less than 200 and 100 IU/mL at the last follow-up, respectively. Two patients developed HBsAg seroconversion at year 2 and 6. No serious adverse events were reported.

Conclusion: Prolonged, uninterrupted entecavir therapy is an effective and safe treatment for CHB patients.

Prevalence of depressive and anxiety disorders and validation of the Hospital Anxiety and Depression Scale as a screening tool in axial spondyloarthritis patients

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Objective: To determine the effects of major depression on various disease assessment tools in spondyloarthritis (SpA) patients.

Methods: Overall, 160 Chinese SpA patients were recruited. Current major depressive disorder (MDD) was diagnosed by a psychiatrist using the Chinese-bilingual Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, 4th edition (CB-SCID). Socio-demographic parameters, Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Bath Ankylosing Spondylitis Functional Index (BASFI), and Ankylosing Spondylitis Disease Activity Score (erythrocyte sedimentation rate- or C-reactive protein-based: ASDAS-ESR or ASDAS-CRP) were compared using univariate analyses and variables with significant differences ($P < 0.1$) were used as independent variables in multivariate regression models using BASDAI, BASFI, and ASDAS-CRP/ASDAS-ESR as dependent variables.

Results: A total of 17 patients were diagnosed with current MDD. BASDAI, ASDAS-ESR, and BASFI were associated with current MDD in univariate analyses ($B=1.69$, $P \leq 0.01$; $B=0.45$, $P=0.08$; $B=2.13$, $P=0.001$, respectively). No association was found in univariate analysis between ASDAS-CRP and current MDD. Multivariate analyses adjusting other clinical variables showed only BASDAI was associated with current MDD ($RC=1.33$; $P=0.01$). BASFI was associated with current MDD in multivariate analysis when BASDAI was not included in the independent variables ($RC=1.57$; $P=0.02$).

Conclusion: The self-rating disease assessing tool BASDAI is more affected by current MDD when compared to ASDAS in SpA patients. Current MDD also has effect on BASFI. As a semi-objective tool, ASDAS might be a better way in assessing disease activities.

The socio-economic impacts on household income, employment status, need for public assistance, and marriage opportunities in patients with spondyloarthritis: a comparison with the Hong Kong population

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Objectives: Our goal is to assess the impacts of spondyloarthritis (SpA) on household income, employment status, need for public assistance, and marriage opportunities by comparing the disease group with the Hong Kong population. We also aimed to identify the potential predictors for lower monthly household income in SpA patients.

Methods: A total of 160 patients were involved in the study. Age- and sex-categorised household income, employment status, need for public assistance, and marriage opportunities were compared between SpA patients and the Hong Kong population. Baseline characteristics were compared between SpA patients with a monthly household income above or below the median Hong Kong Island population monthly household income. Univariate regression analyses were performed using a natural log (ln) of monthly household income as the dependent variable and baseline characteristics with a significant difference ($P < 0.1$) as independent variables. A multivariate regression model was built up using ln (monthly household income as the dependent variable) and factors with a significant difference ($P < 0.1$) in univariate analyses as independent variables.

Results: SpA patients in the older group were found to have lower household income, decreased marriage opportunities, a greater percentage of unemployment, and a need for public assistance. Monthly household income was positively associated with tertiary education ($B = 0.19$, $P = 0.03$) and negatively associated with Modified Stoke Ankylosing Spondylitis Spinal Score ($B = -0.26$, $P = 0.02$), living alone ($B = -0.18$, $P = 0.03$), and the presence of medical problems ($B = -0.19$, $P = 0.03$).

Conclusion: Having SpA has a significant impact on various socio-economic factors. The impacts appeared to increase in the older age-group. Disease severity is one of the major factors that predict a lower household income.

Generation of neurons from LAMP2-deficient iPSCs for modelling lysosomal dysfunction-induced neuropathogenesis

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Introduction: Lysosomes are organelles responsible for the degradation of obsolete cellular constituents and extracellular materials. Emerging body of evidences shows the association between lysosomal dysfunction and neurodegenerative diseases. The LAMP2 protein has been reported to help in maintaining lysosomal membrane integrity. Clinically, primary LAMP2 deficiency is associated with the Danon disease, in which affected individual may manifest severe cardiomyopathy and certain degree of mental retardation. Owing to the difficulties in obtaining and culturing human neurons, the pathological role of LAMP2 deficiency in the neural injury remains unclear. This study was to use the cortical neurons derived from LAMP2-deficient induced pluripotent stem cells (iPSCs) to evaluate the effects of lysosomal instability on neuronal dysfunction

Methods: Generation of iPSCs from a patient with *LAMP2* gene mutation has been performed. The resultant iPSCs have been differentiated into cortical neurons. Western blot and immunofluorescence analysis confirmed the deficient LAMP2 production and detected the expression level of lysosomal enzyme of the patient-specific iPSCs-derived neurons.

Results: The LAMP2-deficient neurons showed leakage of the lysosomal enzyme-cathepsin L.

Conclusion: We suggest that lysosomal membrane instability may involve in the LAMP2-deficiency-induced neuronal dysfunction.

Circulating fibroblast growth factor 21 levels predict progressive kidney disease in subjects with type 2 diabetes and normoalbuminuria

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Introduction: Elevated fibroblast growth factor 21 (FGF21) levels have been suggested from cross-sectional studies, as an indicator of subclinical diabetic nephropathy. We investigated whether serum FGF21 was predictive of the development of diabetic nephropathy.

Methods: Baseline serum FGF21 levels were measured in 1136 Chinese type 2 diabetic subjects recruited from the Hong Kong West Diabetes Registry. The role of FGF21 in predicting decline in estimated glomerular filtration rate (eGFR) over a median follow-up of 4 years was analysed using Cox regression analysis.

Results: At baseline, serum FGF21 levels increased progressively with eGFR category (P for trend <0.001). Among 1071 subjects with baseline eGFR of ≥ 30 mL/min/1.73 m², serum FGF21 levels were significantly higher in those with eGFR decline during follow-up (n=171) than those without decline (n=900) [P<0.001]. In multivariable Cox regression analysis, serum FGF21 was independently associated with eGFR decline (hazard ratio [HR]=1.21; 95% confidence interval [CI], 1.01-1.43; P=0.036) even after adjustment for baseline eGFR. In a subgroup of 559 subjects with baseline eGFR of ≥ 60 mL/min/1.73 m² and normoalbuminuria, serum FGF21 level remained an independent predictor of eGFR decline (HR=1.36; 95% CI, 1.06-1.76; P=0.016). Integrated discrimination improvement suggested that the inclusion of baseline serum FGF21 improved the prediction of eGFR decline over hypertension alone (P=0.013).

Conclusions: Elevated serum FGF21 levels may be a useful biomarker for predicting kidney disease progression especially in early stages of diabetic nephropathy.

Thymomatous myasthenia gravis in Hong Kong Chinese

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Introduction: Myasthenia gravis (MG) is an important autoimmune disease causing generalised weakness and even mortality. It is associated with pathological abnormalities of the thymus gland in about 80% to 85% of patients. The reported frequencies of thymoma in MG patients is about 10% to 20% and thymomatous MG (T-MG) was in general believed to be more severe and associated with worse prognosis. We studied the frequency, clinical features, and long-term outcome of T-MG in our population.

Methods: Records of generalised MG (gMG) patients cared in Queen Mary Hospital (QMH) and followed up in QMH neurology clinic from 1997 to 2012 were reviewed.

Results: Overall, 123 gMG patients were studied and 45 (36.6%) of them had thymoma. Of these 45 patients, 42 (93.3%) T-MG had thymectomy compared to only 44% in non-thymomatous gMG (NT-MG) patients. There was no significant difference in the mean age of onset in T-MG (45.14 years) compared to NT-MG patients (45.04 years), but early onset disease (<40 years of age) was more common in NT-MG (odds ratio [OR]=2.41, P=0.03). Univariate analysis revealed that T-MG patients had higher frequencies of anti-AChR Ab seropositivity (100% vs 81.2%, P=0.002) and anti-striated muscle Ab seropositivity (70.4% vs 30.6%, P=0.002), and lower frequency of other autoimmune diseases (6.7% vs 25.3% P=0.014) than NT-MG, but were indifferent in sex, onset and worst clinical severity, presence of bulbar symptoms, history of MG crisis, co-morbidity status, and use of immunosuppressive therapies. There was no difference in long-term clinical outcome between T-MG and NT-MG patients. In multivariate analysis, T-MG was found to be associated with higher frequency of anti-striated muscle Ab (OR=3.97, P=0.029) and lower frequency of other autoimmune diseases (OR=0.18, P=0.049).

Conclusion: Frequency of thymoma in local Chinese MG patients appeared to be higher than MG patients in other regions of the world. Overall the clinical features appeared similar between T-MG and NT-MG patients. With prompt and proper treatment, the long-term prognosis is also similar between the two groups.

Lipocalin 14: a novel adipokine that protect mice against diet-induced diabetes via glycerol metabolism

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Introduction: Lipocalin 14 (LCN14) is a novel adipokine. In mouse, development of diabetes is associated with severe suppression of LCN14 in high-fat diet (HFD) and genetic-induced obese (db/db) mice. Restoration of LCN14 expression in HFD-treated mice by using recombinant adeno-associated virus (rAAV) gene delivering system could alleviate diet-induced glucose intolerance and insulin resistance in the mice potentially via inhibition of glycerol translocation from adipose tissue to liver.

Methods: Wildtype C57/BL6N mice were fed with either HFD or chow diet, and then infected with rAAV overexpressing LCN14 protein. Glucose, pyruvate, and insulin tolerance tests were performed in week 7, 8, and 9 of HFD treatment, respectively. Serum lipid profile was evaluated bi-weekly. The mice were sacrificed and major metabolic organs were collected for further molecular and biochemical analysis.

Results: Overexpressing LCN14 could lower serum glycerol level, improve glucose tolerance, pyruvate tolerance, and insulin sensitivity in insulin-independent manner without changes of body weight and percentage of fat in mice.

Conclusion: LCN14 deficiency is likely to contribute to development of diabetes in obese mice. Overexpression of LCN14 in diet-induced obese mice can lower production and efflux of glycerol in adipose tissues and hence, lead to inefficient gluconeogenesis in the liver. LCN14 may be a potential therapy for type 2 diabetes treatments.

Mediterranean-style diet is associated with reduced blood pressure variability and subsequent stroke risk in patients with coronary artery disease

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Introduction: The Mediterranean-style diet is widely advocated for the prevention of cardiovascular diseases. Meanwhile, blood pressure variability (BPV) is a novel risk factor for cardiovascular disease. It is unknown whether dietary pattern plays a role in modulating BPV.

Methods: We prospectively followed up 274 consecutive patients with stable coronary artery disease (CAD). The Mediterranean diet score (MDS) was derived for all individuals upon recruitment, blood pressure was measured during each subsequent clinic visit and the visit-to-visit BPV was calculated. The occurrence of major adverse cardiovascular events (MACEs) and all-cause mortality was monitored.

Results: After a mean follow-up of 77 ± 12 months, 16.1% of the study population developed MACEs. About 11.3% died from all causes. Patients who developed MACEs or all-cause mortality had a greater systolic BPV compared to those who did not develop an adverse event. Patients who developed a MACE had a lower MDS and further analysis revealed those who developed a stroke had a lower MDS compared with those who did not develop a stroke, but there were no significant differences in MDS between CAD patients with or without subsequent acute coronary syndrome, cardiovascular, or all-cause mortality. After adjusting for confounding variables, a high MDS was an independent predictor for low systolic BPV (B -0.74 , 95% confidence interval [CI], -1.27 to -0.21 ; $P < 0.01$) and was noted to be protective against subsequent development of stroke (hazard ratio = 0.48 ; 95% CI, $0.24-0.94$; $P = 0.03$).

Conclusion: Among patients with CAD, a higher MDS is associated with a lower visit-to-visit BPV and with lower stroke risk.

miR-296-5p and miR-874-3p inhibited HCC cell proliferation by targeting on PIN1

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Introduction: PIN1 is a peptidyl-prolyl cis/trans isomerase (PPIase) that regulates multiple signalling pathways to control cell fate. It is found to be up-regulated in hepatocellular carcinomas (HCCs), leading to increased cell proliferation and inhibition of apoptosis. However, the regulation of PIN1 remains poorly defined. MicroRNA (miRNA) plays a pivotal role in carcinogenesis by regulating tumour suppressor genes and oncogenes via targeting the 3'UTR of their encoded mRNAs. Therefore, we aimed at identifying miRNAs regulating PIN1 expression, and determining the contributions of these miRNAs to tumourigenesis through PIN1.

Methods: Potential miRNAs regulating PIN1 were identified through sequence prediction using the online database TargetScan Release 6.2. Western immunoblotting and quantitative reverse-transcription polymerase chain reaction (qRT-PCR) were used to assess PIN1 expression in HCC cells with over-expression of identified miRNAs. Dual-Luciferase Reporter Assay was conducted to examine the direct interaction between the identified miRNAs and PIN1 3'UTR. The tumour suppressive role of the identified miRNAs in HCC cells was investigated by MTT cell proliferation assay, colony formation assay, and Annexin V apoptosis detection kit. To investigate the correlation between the levels of PIN1 and the identified miRNAs, qRT-PCR was used to determine the mRNA levels of PIN1 and the identified miRNAs in 20 pairs of normal liver tissue and HCC.

Results: We identified miR-296-5p and miR-874-3p as important negative regulators of PIN1. Consequently, over-expression of miR-296-5p and miR-874-3p enhanced cellular apoptosis, and inhibited cell growth and colony formation in HCC cells. Furthermore, the cellular functions of miR-296-5p and miR-874-3p were abrogated by over-expression of PIN1. Importantly, the expression of PIN1 was negatively correlated with that of miR-296-5p and miR-874-3p in HCC tissues, consistent with the regulatory role of miR-296-5p and miR-874-3p on PIN1 expression.

Conclusion: Taken together, our data showed that miR-296-5p and miR-874-3p controlled cell proliferation and apoptosis in HCC by regulating PIN1 expression.

Patient characteristics of those willing to join a local hospital osteoporotic fracture prevention programme

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Introduction: Osteoporosis is a common and potentially debilitating disease. With ageing population, the incidence of osteoporosis is anticipated to increase tremendously. Treatment of osteoporotic fracture is costly. Osteoporosis has been underestimated both by health care professionals and patients.

Objective: To study patient characteristics of those at risk of osteoporotic fracture.

Methods: Patients seen in Out-Patient Department with a history of osteoporosis/osteopenia or fulfilling any one of the referral criteria—(A) postmenopausal women, (B) sex hormone deficiency, (C) two or more of the risk factors including (i) steroid users taking prednisolone 5 mg daily or more for more than 3 months, (ii) low body mass index (BMI) of less than 18 kg/m², (iii) chronic smoker, (iv) alcoholic taking more than 3 units of alcohol per day—were referred to the osteoporotic fracture prevention programme for assessment during the period November 2012 to December 2013. Patients with osteoporosis/osteopenia would be enrolled into the prevention programme including education and physiotherapy training on voluntary basis. The programme adherence rate, attendance rate, patient satisfaction questionnaire score, and number of patients with newly diagnosed osteoporosis/osteopenia due to this screening programme would be collected.

Results: Overall, 27 female patients joined the programme with a mean age of 69 (range, 52-83) years. All were postmenopausal women except one patient. The mean body weight and body height was 53.4 kg (range, 38.9-64.8 kg) and 152.6 cm (range, 144.5-164.0 cm), respectively. The mean BMI was 22.9 (range, 18.2-27.6) kg/m². Three (11.1%) patients were ex-smokers and 24 (88.9%) were non-smokers, and all patients were non-drinkers. Three (11.1%) patients had a history of glucocorticoid use; 11 (40.7%) patients had a previous history of fracture. The mean T score of femoral neck was -2.2 (range, -0.7 to -3.7) and spine was -1.8 (range, -0.2 to -3.6). Twelve (44.4%) patients were already on anti-osteoporosis agents (7 on bisphosphonate, 3 on denosumab, and 2 on strontium) upon assessment. Both the programme adherence rate and attendance rate were 100%. Of the patients, 70.4% (19 out of 27) had finished the patient satisfaction questionnaire and the mean satisfaction score was 17.5 out of 20 (range, 12-20). Five (18.5%) patients were newly diagnosed to have osteopenia; one (3.7%) patient was newly diagnosed to have osteoporosis and started bisphosphonate.

Conclusion: Osteoporosis or osteopenia is a common problem that can be totally asymptomatic but with significant morbidity or mortality resulted from fracture. Our patients are satisfied with the programme. Self-management programme has been shown to empower patients with chronic illness. Further analysis of the pre- and post-rehabilitation patient parameters will be carried out to find out the effectiveness of such programme.

Arsenic trioxide suppresses tumour growth in squamous cell lung carcinoma

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Introduction: Squamous cell lung carcinoma (SCC) belongs to the second most common subtype in non-small-cell lung carcinoma. Recently, doublet chemotherapy regimens remain the cornerstone of first-line systemic treatment. Therefore, new therapeutic approach is urgently needed. Arsenic trioxide (ATO) is a traditional Chinese medicine which has multiple anti-cancer mechanisms including apoptosis. ATO has been used clinically in acute promyelocytic leukaemia. ATO has been shown to induce apoptosis in lung adenocarcinoma in vitro and in vivo. The aim of this study was to determine the anti-cancer effect of ATO in SCC.

Methods: Two SCC cell lines were obtained: SK-MES-1 and SW900. Cell cycle arrest, phosphatidylserine externalisation, mitochondrial membrane depolarisation, and reactive oxidative species level were analysed by flow cytometry. Cell viability and protein expression were determined by MTT assay and Western blot, respectively. Effect of ATO was demonstrated by SK-MES-1 xenograft model in vivo.

Results: Upon ATO treatment, G2/M arrest was noted in both cell lines while level of phosphatidylserine externalisation and mitochondrial membrane depolarisation were increased in SK-MES-1 cells only. Hydrogen peroxide was raised in SK-MES-1 cells while decreased in SW900 cells. Superoxide level was decreased in SK-MES-1 cells while unaltered in SW900 cells. Sustained Erk activation was observed in SK-MES-1 cells only. The expression level of p-p38 was increased in SK-MES-1 cells while it was decreased in SW900 cells. Cleaved caspase 3 was elevated in both cell lines. Cleaved PARP was increased in SK-MES-1 cells while Bak was upregulated in SW900 cells. Anti-apoptotic protein XIAP was decreased in both cell lines, while Bcl-2 was downregulated in SK-MES-1 cells only. Transcriptional factor protein, E2F-1, was decreased in both cell lines, while RRM1 and thymidylate synthase were decreased in SK-MES-1 cells only. ATO (7.5 mg/kg) decreased the tumour size in SK-MES-1 xenograft in vivo.

Conclusion: ATO induced apoptotic and anti-proliferative effects in SCC in vitro and in vivo.

Defining criteria for rheumatoid arthritis patient-derived Disease Activity Score (PDAS) that correspond to Disease Activity Score 28 (DAS28) and Clinical Disease Activity Index (CDAI) Based Disease Statuses And Response Criteria

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Introduction: Patient-derived Disease Activity Score with ESR (PDAS1) and without ESR (PDAS2) in rheumatoid arthritis (RA) are validated patient-reported outcome measures of disease activity and correlate highly with Disease Activity Score 28 (DAS28) and Clinical Disease Activity Index (CDAI). The purpose of this study was to develop and examine their performance of status and responder criteria.

Method: Data from 299 RA patients (originally used to develop PDAS) were analysed using receiver operator characteristic (ROC) curves to determine optimal cut-points for PDAS corresponding to DAS28 and CDAI criteria for remission, low-, medium- and high-disease activities. Data from 56 RA patients started on disease-modifying drugs before and 6 months after treatment were used to determine PDAS thresholds corresponding to the European League Against Rheumatism (EULAR) good or moderate responses. Agreement was assessed with kappa statistics.

Results: Key cut-points for PDAS1&2 were 3.5, 4.5, 4.8, and 3.8, 4.6, 5.0 respectively. Area under ROC curves ranged from 0.89 to 0.95. Sensitivities ranged from 79% to 99%, and specificities from 61% to 89%. Moderate-to-good agreement with DAS28 categories was observed: respectively, $k=0.44$ and 0.31 for PDAS1&2. Corresponding agreements with CDAI were $k=0.3$ and 0.4 . Crucially, these agreements were comparable to those of CDAI and DAS28 ($k=0.54$). The criteria that corresponded to EULAR moderate and good response were 0.4, 0.8 for PDAS1 and 0.3, 1.2 for PDAS2. Area under the ROC curve ranged from 0.88 to 0.93. Sensitivities ranged from 72% to 100% and specificities from 77% to 94%. Agreement of DAS28 response with PDAS1&2 were $k=0.46$ and 0.38 respectively. Again, these were comparable to the agreement between DAS28 and CDAI in this patient group ($k=0.55$).

Conclusion: Disease activity and treatment response for PDAS1&2 have comparable agreement to standard criteria DAS28 and CDAI. PDAS has potential use in routine practice and research.

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Introduction: We have previously presented data regarding the use of neoadjuvant chemotherapy (NAC) to treat locally advanced breast cancer (LABC) in Hong Kong West Cluster. For patients without HER2 gene amplification, NAC consists of standard chemotherapy containing an anthracycline and a taxane usually given sequentially. We hypothesised that high-risk patients with aggressive tumour phenotypes or those who failed to respond to the standard chemotherapy would benefit from a more intense regimen. From 2012 we prospectively recruited such patients undergoing NAC and administered a third-generation regimen containing an anthracycline, taxane, and cyclophosphamide given concurrently with growth factor support.

Method: Before NAC was started, all patients were mandated to have positron emission tomography/computed tomography (CT) or CT with contrast to demonstrate the absence of distant metastasis. Pre-treatment tumour biopsies have immunohistochemistry for ER, PR, HER2 and Ki67 performed by reference labs. Suspicious axillary lymph nodes have fine-needle aspiration performed to assess for metastasis. High risk was defined as either ER/PR+ HER2 -ve and highly proliferative (Ki67 >30% or >10 active mitosis per high-power field) or ER/PR/HER2 -ve (TN); this constituted risk stratification according to tumour biology. The response to tumour was assessed every cycle by the oncologist and assessed by bedside ultrasound every 2 to 4 cycles. For those receiving standard NAC, if there was lack of tumour shrinkage or they show clinical progression, they are offered cross-over to the high-risk regimen at the discretion of the oncologist, with response guided. The chemotherapy was planned for 6 to 8 cycles depending on the tolerability of the patient. After completion of NAC, radiological reassessment to demonstrate clinical disease control was performed. Surgical intervention was delivered according to international guidelines and final pathology was reported according to international standards.

Results: Since 2012, 19 patients were treated according to this protocol; their mean age was 48 years, with 74% pre-menopausal. Of these patients, 84% were intrinsically high risk with the rest being poor responders. The proportion of TN breast cancer was 37%. Disease control rate was 95% and one patient developed clinical progression during NAC. The median cycles of NAC delivered was 6 and there was no treatment-related mortality. Breast conservation was performed in 16% of the patients.

Conclusion: Adapting a risk-stratified and response-guided approach for neoadjuvant therapy can enhance the rate of tumour control and potentially deliver higher breast conservation rate for LABC.

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Intracerebral haemorrhage (ICH) is a devastating form of stroke which is characterised by breakdown of blood vessels within the brain parenchyma. ICH has a mortality rate much higher than that of ischaemic stroke and it could lead to poor neurological outcomes including motor deficits and cognitive impairment. At present, the therapeutic options for ICH remain limited. As many studies have shown that the activation of oxidative pathways and inflammatory pathways are the important causes of brain damage after ICH, the present study aimed to investigate whether melatonin—a potent antioxidant and free-radical scavenger with strong anti-inflammatory actions—could provide neuroprotection after ICH. ICH was induced in rats by intrastriatal injection of collagenase type IV. Repetitive melatonin intraperitoneal injections were given to the rats at 2 h, 24 h, and 48 h after ICH, and they were sacrificed at 72 h. The beneficial effects of melatonin on neurological deficits after ICH were assessed by rotarod test and neurological deficit scoring system. Further investigations will be focused on the effectiveness of melatonin in alleviating inflammation after ICH as well as other possible mechanisms.

Role of gut microbiota in the pathogenesis and progression of atherosclerosis in mice

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Introduction: Gut microbiota is involved in both onset and progression of obesity. Obesity is associated with several cardiovascular diseases among which atherosclerosis is the leading cause of morbidity and mortality. However, the role of gut microbiota in obesity-related cardiovascular complication remains to be explored. We found that the faecal amount of *Akkermansia muciniphila* (Akk), a beneficial gut bacteria, decreased in apoE-deficient mice fed a high-fat-high-cholesterol (HFHC) diet, a murine atherosclerotic model. This study investigated the role of Akk in the pathogenesis and progression of atherosclerosis.

Method: Either phosphate buffer saline (PBS) or Akk were daily oral gavaged to apoE-deficient mice fed a HFHC diet for 8 weeks. During the experimental periods, body weight and fat mass were measured weekly. After 8 weeks, mice were sacrificed, atherosclerotic lesions were analysed by aortic root H&E staining and en face aorta Oil Red O staining.

Results: Daily oral gavage of Akk decreased body weight and fat mass of apoE-deficient mice in spite of being fed a HFHC diet. Akk treatment significantly reduced atherosclerotic lesion without changing the major gut microbiota composition. Serum total cholesterol, triglyceride, high- and low-density lipoprotein levels were not significantly affected by Akk treatment; however, serum lipopolysaccharide decreased dramatically. Increased adipose tissue lactate levels were commonly found in obesity. We found that lactate levels in mesenteric adipose tissue were reduced upon Akk treatment. Lactate-induced pro-inflammatory responses by stimulating iNOS and IL1 β expressions in macrophages. Lactate can serve as a toxic metabolite accelerating inflammation in obesity and cardiovascular diseases.

Conclusion: Our results demonstrated that Akk can decrease atherosclerotic lesions and possibly by decreasing lactate levels and inflammation in murine model.

Localisation and functional study of synaptogyrin-3 (SYNGR3) on neuronal dopaminergic system

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Introduction: Synaptogyrin-3 (SYNGR3) is an integral synaptic vesicle protein in the synaptogyrin family. Among the three isoforms of synaptogyrin (SYNGR1-3), SYNGR3 is specifically expressed in the brain. However, the physiological function of SYNGR3 in neurons is currently unknown. Recent evidence has shown that SYNGR3 can physically interact with dopamine uptake transporter (DAT). Intracellular dopamine (DA), if not properly sequestered, will undergo auto-oxidation leading to oxidative damages. Therefore, we hypothesise that SYNGR3 has a functional role in dopaminergic neurons to facilitate DA re-take for vesicle packaging and DA recycling. In this study, we investigated the intracellular localisation and protein-protein interaction between SYNGR3 and DAT, and determined how expression of SYNGR3 in neurons affects DA uptake efficiency.

Methods: Subcellular localisation of SYNGR3 and DAT in mouse brain striatum was visualised by immunogold electron microscopy (EM). Protein-protein interaction between SYNGR3 and DAT was determined by immunoprecipitation and Western blotting. The functional role of SYNGR3 on DA uptake was determined by [3H]-DA uptake assay in human SH-SY5Y neuroblastoma cells after overexpressing SYNGR3.

Results: Immunogold EM revealed that SYNGR3 was co-localised with DAT in the striatal synaptic termini. Immunoprecipitation of DAT using anti-DAT antibody resulted in co-precipitation of SYNGR3 from mouse brain striatal lysates, and vice versa. SH-SY5Y cells overexpressing SYNGR3 caused significant increase in overall DA uptake activity as compared with empty-vector controls.

Conclusion: Our findings demonstrated that overexpressing SYNGR3 in neuronal cells increased DA uptake efficiency, possibly via protein-protein interaction with DAT. Striatum is enriched with dopaminergic synapses projected from the midbrain and cortex. Co-localisation of SYNGR3 and DAT in striatal synapses may have important functional role to regulate DA homeostasis in motor movement and cognitive functions.

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***Helicobacter pylori* infection alters human gastric microbiota and bacterial diversity**

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Introduction: *Helicobacter pylori* (HP) is well studied as a type I carcinogen, but the distribution and significance of other bacteria in the human stomach remain poorly characterised. This study aimed to characterise the gastric microbiota in individuals with and without HP infection, to determine the changes in microbiota after HP eradication, and to demonstrate the alterations of gastric microbiota in individuals with different histological stages of gastric carcinogenesis.

Methods: Endoscopic gastric biopsies were obtained from patients with no ulcer or tumour found on gastroscopy, no prior HP eradication or recent antibiotics treatment. HP infection status was determined by rapid urease test and histology examination. Bacterial 16S rRNA genes were extracted from gastric biopsies and sequenced on next-generation sequencing platforms. Operational taxonomic unit clustering, diversity indices calculation, taxonomic classification, principal coordinates analysis, and statistical analyses were performed after quality control and raw sequence processing. Hierarchical clustering based on weighted UniFrac distance of samples using Ward's algorithm was implemented.

Results: Overall, 35 patients were studied including: (1) 13 HP-infected patients, (2) 14 HP-negative individuals matched for age and sex, and (3) 8 HP-infected patients who had serial endoscopic biopsies taken before and after receiving antibiotics for HP. Hierarchical clustering of samples in the first two groups demonstrated two cluster groups: one cluster (cluster A) contains mostly HP-negative samples (n=37) while the other cluster (cluster B) exclusively comprised HP-positive samples only (n=16). Cluster A had markedly greater microbial species diversity with an average Shannon diversity index of 4.08 (standard deviation [SD], 0.50) comparing to 1.95 (SD, 0.46) of cluster B (P<0.01). Same analyses were applied to group 3 to compare the gastric microbiota in HP-infected patients before and after antibiotics treatment. Almost all post-treatment samples had increased bacterial diversity. As the relative abundance of HP decreased in the post-treatment group, non-HP *Proteobacteria* (P<0.01), *Fusobacteria* (P<0.01), and *Bacteroidetes* (P<0.01) were found to be increased. To assess the alterations of gastric microbiota at different stages of gastric carcinogenesis, we recruited 8 patients who had HP gastritis, 9 who had intestinal metaplasia, 7 who had gastric carcinoma, and 8 normal controls. Hierarchical clustering of these samples was mainly determined by the presence of HP but not by histological stages. Nonetheless, cancer patient samples had reduced bacterial diversity comparing to the other histological groups.

Conclusion: *H pylori* colonisation results in alteration in gastric microbiota and reduction in bacterial diversity. These changes could possibly be restored by antibiotics treatment. The change in gastric microbiota associated with disease progression is not as prominent as the effect of *H pylori* infection.

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Involvement of MAPK and AMPK signalling pathways in cigarette smoke-induced inflammation in human AC16 cardiomyocytes

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Introduction: Cigarette smoke (CS) is the major risk factor of cardiovascular diseases. There is increasing evidence showing oxidative stress and inflammatory responses may play roles in the pathophysiological process; however, the underlying mechanism on CS-induced inflammation is currently unclear. The aim of this study was to investigate whether mitogen-activated protein kinase (MAPK) and AMP-activated protein kinase (AMPK) signalling pathways are involved in CS-induced inflammatory responses in human AC16 cardiomyocytes in vitro.

Methods: The AC16 cell line was cultured in DMEM/F12 containing 12.5% fetal bovine serum, in a CO₂ incubator at 37°C. When cells reached 70% confluence, the medium was replaced with a medium consisting of 1% fetal bovine serum 24 hours before treatment. Cigarette smoke medium (CSM) was prepared by bubbling smoke from two cigarettes into 20 mL serum-free medium, which was regarded as 100%. Cells were pretreated with MAPK pathway inhibitors including U0126 (an ERK1/2 inhibitor), SB203580 (a p38 inhibitor) and SP600125 (a JNK inhibitor) and AMPK inhibitor Compound C for 30 minutes before 2% CSM was added and incubated for an additional 24 hours. Supernatant was collected for determination of interleukin (IL)-8 and IL-6 by enzyme-linked immunosorbent assay. Cell lysates were collected for Western blot analysis.

Results: CSM caused dose-dependent elevation of IL-8 and reduction of IL-6 in the supernatant of cardiomyocytes. MAPK inhibitors attenuated CSM-induced elevation of IL-8 release but no effect on CSM-induced reduction of IL-6 release. On the other hand, Compound C abolished both CSM-induced IL-8 elevation and IL-6 reduction. Furthermore, CSM increased protein expression of anti-oxidative stress enzyme heme oxygenase 1 (HO-1) and quinone oxidoreductase 1 (NQO1), suggesting the existence of a defensive mechanism in cardiomyocytes.

Conclusion: These findings suggest that MAPK but not AMPK have a differential role in controlling CSM-induced IL-8 and IL-6 release via oxidative stress in human cardiomyocytes in vitro.

Leucine-rich repeat kinase-2 (LRRK2) R1441G knockin mice are more susceptible to rotenone toxicity

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Introduction: LRRK2 is linked to synapse functions. However, the pathogenic mechanism of LRRK2 mutation in striatal dopamine (DA) homeostasis and mitochondria dysfunction is unknown. We aimed to assess the susceptibility of LRRK2 R1441G knockin (KI) mice against (1) striatal DA uptake deficit, (2) locomotor inactivity, and (3) dopaminergic neuronal cell death, as induced by rotenone (mitochondrial complex-I inhibitor).

Methods: Cell viability of primary DA neurons from R1441G knockin mice and wild-type (WT) littermates were compared by immunohistochemistry after rotenone exposure. [³H]-DA uptake in isolated striatal synaptosomes from young (3 months old) KI mice incubated with rotenone was compared with WT littermates. Locomotor activity in open-field test after chronic (20 weeks) oral gavage of rotenone was also assessed.

Results: Without rotenone, R1441G mutant mice showed no overt phenotype. However, synaptosomes from young (3 months old) mutant mice exhibited lower DA uptake when incubated with rotenone (100 nM), compared with WT controls. Number of DA neurons in mutant culture with rotenone exposure (5 nM) was significantly lower. Also, chronic exposure to rotenone (5 mg/kg, twice per week orally) for 20 weeks caused significantly lower locomotor activity in mutant mice compared with the WT controls.

Conclusion: Despite the apparent lack of abnormal phenotype in our *LRRK2*^{R1441G/R1441G} KI mice, current results show that a differential functional susceptibility can still exist. DA neurons and locomotor activity appeared normal in these KI mice under normal condition. However, as what we have shown recently, they are more liable to DA depletion and locomotor inactivity. This differential susceptibility against rotenone toxicity of the mutant mice suggests that LRRK2 R1441G mutation may be a predisposing genetic factor in synaptic energy deficiency leading to early striatal synaptic dysfunction, and later nigrostriatal DA cell death in LRRK2-associated Parkinson's disease. How pre-synaptic defects and abnormal DA homeostasis lead to neuronal cell death associated with LRRK2 mutations require further investigations.

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Post-BMT survival analysis of acute myeloid leukaemia patients in Hong Kong

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Introduction: Acute myeloid leukaemia (AML) is one of the most lethal cancers in Hong Kong. It is a group of highly heterogeneous diseases with distinct cytogenetic, and prognostic features sharing in common increase in myeloblasts (>20%) in bone marrow or circulation. Intensive chemotherapy and allogeneic bone marrow transplantation (BMT) are the mainstays of treatment, though the outcome is often unsatisfactory. By observing post-BMT relapse-free (RF) period and overall survival (OS), we may be able to distinguish what factors are important in affecting the outcome of receiving BMT.

Methods: Clinical information of 680 patients who were aged ≤ 60 years and diagnosed with AML from 2003 were collected and Kaplan-Meier survival analysis was performed.

Results: Patients of good/intermediate risk had a similar RF rate of around 65%, while those of poor risk remained a low rate of 30%. OS of good/intermediate risk stayed close to each other at a rate of 60% and 55%, while poor risk only had 20%. Patients received BMT at first or second complete remission (CR1/CR2) both had a RF rate of 55% and OS rate of 50%. Those whose haematopoietic stem cells were from siblings had a RF rate of 70% while those from HLA-matched unrelated donors (MUD) only had 50%. However, they both shared the same OS rate of 50%. Patients achieving CR1 with one or two courses of chemotherapy have RF rate of 60% while those received more than two courses only have 40%, although those achieved CR1 with only one course of chemotherapy have OS of 50%, while those with two or more than two courses have only 40%. People who had high-dose AraC (HDAC) involved consolidation before BMT had a RF rate of 60% and OS rate of 50%, while people who had consolidation with no HDAC involved or simply no consolidation given had only RF rate of 40% and OS rate of 30%.

Conclusion: Patients with poor-risk cytogenetics conditions remain generally poor even after BMT, meaning BMT may not be the best option. Having BMT at CR1 or CR2 does not seem to have a big difference as in RF period or OS. Sources of stem cells from sibling may have higher relapse rate than MUD, though this does not affect the OS. Achieving CR1 with one or two more courses of induction has slightly better RF than more than two courses, though the OS of having received only one course of induction is better than that of two or more courses. Receiving HDAC consolidation before BMT seems to have a better outcome either in terms of RF or OS.

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A prospective study of the effect of telbivudine on renal function in chronic hepatitis B patients with mild-to-moderate renal impairment

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Background: Although some antivirals used in chronic hepatitis B (CHB) patients can worsen the renal function, telbivudine seems to have renal positive effects. We aimed to assess the renal function after switching lamivudine to telbivudine.

Methods: From August 2013, we recruited CHB patients on either lamivudine plus tenofovir or lamivudine plus adefovir with estimated glomerular filtration rate (eGFR) of 30-89 mL/min/1.73 m². Renal function, cystatin C, liver biochemistry, serum hepatitis B virus (HBV) serology, HBV DNA, creatinine kinase (CK), phosphate, 24-hour urinary protein, and spot urine glucose were prospectively monitored at every 12 weeks for 2 years. 24-Hour urinary phosphate levels were tested every 24 weeks. Changes of eGFR were compared by paired Student's *t*-tests.

Results: Thirteen patients (12 male and 1 female) with a mean age of 56.4±5.8 years were recruited. At baseline, 13 were hepatitis e antigen negative, 3 (23%) had diabetes mellitus, and 4 (30.8%) had hypertension. Mean nucleotide analogues exposure was 5.6±1.6 years. Four (30.7%) patients had moderate renal impairment (eGFR, 30-59 mL/min/1.73 m²). The mean eGFR calculated by modification of diet in renal disease and a combined cystatin C and serum creatinine based eGFR equation showed a 15.8% (P=0.04) and 10.9% (P≤0.001) improvement at month 12. Three (75%) patients' eGFR improved from moderate to mild renal impairment. HBV DNA remained undetectable at month 12. Three patients had asymptomatic raised CK level of <1000 U/L at month 12. One patient required oral phosphate supplement for hypophosphatemia without hyperphosphaturia.

Conclusions: From this interim analysis, telbivudine combined with a nucleotide analogue significantly improves the eGFR in CHB patients with mild-to-moderate renal impairment. CHB patients with pre-existing moderate renal impairment appeared to benefit more from the combination therapy. No serious adverse effects were noted.

Visit-to-visit systolic blood pressure variability predicts renal function decline in patients with ischaemic stroke

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Introduction: Blood pressure variability (BPV) is an established poor prognostic indicator among patients with ischaemic stroke (ISS). Whether visit-to-visit BPV also predicts renal function decline, independent of average blood pressure (BP) control among ISS patients is nevertheless unknown.

Methods: We retrospectively reviewed the renal function tests taken from 461 ISS patients without atrial fibrillation recruited from 2004 to 2008. The average BP and visit-to-visit BPV, as determined by the standard deviation of the systolic and diastolic BP, were recorded over six clinic visits.

Results: The mean age of the population was 70±11 years; 74% of the population had underlying hypertension and 38% had diabetes mellitus. An average of 4±2 yearly measurements of glomerular filtration rate (GFR) was obtained over a mean follow-up of 76±17 months. During this period, 29% of the study population had a decline in GFR and the mean rate of GFR decline was -1.3±7.6% per year. Multivariate analysis revealed that, independent of average systolic BP, diabetic status and other confounding factors, a raised visit-to-visit systolic BPV was an independent predictor of renal function decline (odds ratio=1.03; 95% confidence interval, 1.00-1.07; P<0.05).

Conclusions: Independent of average BP control and diabetic status, visit-to-visit BPV predicts renal function decline among ISS patients.

Targeting sodium/hydrogen exchanger 1 (NHE1) and its upstream activators in acute myeloid leukaemia

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Activation of sodium/hydrogen exchanger 1 (NHE1) and alkali intracellular pH (pHi) in acute myeloid leukaemia (AML) have been associated with the oncogenesis and drug resistance. Activity of NHE1 was controlled by a series of kinases such as PDGFR, PKC, p90-RSK, and ROCK-RhoA. However their relevance to the oncogenic characteristics in AML was unknown. We hypothesised that targeting NHE1 together with its upstream activators might offer a novel and much effective therapeutic strategy in AML.

AML cell lines ML2, Kasumi-1, MOLM-13 and MV4-11 were more sensitive than KG1, NB4, THP-1 and OCI-AML3 to the growth inhibitory effects of NHE1 inhibitor, 5-(N,N-hexamethylene) amiloride (HMA), accompanied with a larger extent of cellular acidification and apoptosis induction in those four HMA-sensitive lines. In search of the upstream activators of NHE1 relevant to AML, the cell lines were treated with specific inhibitors targeting potential NHE1 activators. Both HMA-sensitive and insensitive cell lines were susceptible to the intracellular acidification and growth inhibition by PDGFR and p90-RSK inhibitors. Primary AML samples were treated with inhibitors to NHE1 (n=50), PDGFR (n=50), and p90-RSK (n=36) [concentration: 100 nM to 10 mM] in vitro. Their responses to the growth inhibitory effect of HMA, accompanied by effective pHi reduction (n=10), correlated with that of PDGFR and p90-RSK inhibitors (Pearson $r=0.74$, $P<0.001$ and $r=0.73$, $P<0.001$, respectively). The data supported the proposition that these pathways might be critical in activating NHE1. Furthermore, synergism of anti-leukaemia effects was demonstrated between HMA and PDGFR/p90-RSK inhibitors.

NHE1 plays an important role in AML survival and is activated by PDGFR, p90-RSK or both in a patient-specific fashion. Therefore, combining different inhibitors targeting NHE1 and its upstream activators should be explored as novel therapeutic approach in AMLs.

The role of calreticulin (CALR) in vertebrate haematopoiesis

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Introduction: Calreticulin (CALR) is a multi-functional protein localised in the endoplasmic reticulum (ER), cytoplasm, cell membrane, and extra-cellular matrix. Its major functions are to control protein folding and Ca^{2+} homeostasis in the ER. Recently, mutations in CALR were found in 67% and 88% of essential thrombocythemia (ET) and primary myelofibrosis (PMF) patients without mutations in Janus kinase 2 (JAK2) or thrombopoietin. It is hypothesised that CALR mutations may also act through the important JAK2-STAT5 signalling pathway in pathogenesis of myeloproliferative neoplasm (MPN). However, the precise role of CALR in haematopoiesis remains unknown, which largely prevented our subsequent understanding about the pathogenic mechanism of CALR mutation in MPN. In this study, we made use of the zebrafish model to investigate the hitherto unknown role of *calr* in vertebrate haematopoiesis.

Methods: Expression pattern of zebrafish *calr* was evaluated by whole-mount in-situ hybridisation (WISH) and semi-quantitative reverse-transcription polymerase chain reaction (RT-PCR). To knock-down (KD) and knock-out (KO) of *calr*, morpholinos (MO) and transcription activator like effector nuclease (TALEN) were used. The efficiency of *calr* MO was tested by co-injection of pEGFP-N3-*calr*MO and *calr* MO and the effect of *calr* KD was evaluated by WISH and quantitative RT-PCR (Q-PCR).

Results: *Calr* is highly conserved in vertebrates including zebrafish. Zebrafish *calr* was expressed predominantly in head region during embryonic development. It was also expressed in the hatching gland and axial vasculature at 18-24 hours post-fertilisation (hpf), in caudal haematopoietic tissue at 48 hpf and later in the lateral line system. In adult zebrafish, *calr* highly expressed in muscles, spleen, and kidney tissues. Molecular targeting of *calr* MO was confirmed by the quenching of GFP expression in embryos co-injected with pEGFP-N3-*calr*MO. KD of *calr* resulted in a decrease in expression of genes associated with myeloid lineages at 24 hpf, including *l-plastin*, *mpo* and *mpeg1* and also an increase in expression of *c-myb* (associated with haematopoietic stem cell [HSC]) at 48, 72, and 96 hpf. Results from WISH were confirmed by Q-PCR. To generate stable *calr* knock-out model, two TALEN pairs targeting Exon 1 and Exon 9 of *calr* were designed and their mutagenic activities were shown by restriction fragment length polymorphism assay.

Conclusion: KO of *calr* affected myeloid and HSCs lineages in zebrafish embryos and provide important ground for further understanding of the unknown role of *Calr* in haematopoiesis.

Acknowledgement

This work was supported by the HKU Seed Funding for Basic Research (201401159004) and Zebrafish Core Facility, LKS Faculty of Medicine.

Relationship of pericardial fat with biomarkers of inflammation and haemostasis, and cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis

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Objective: Pericardial fat may increase the risk of cardiovascular disease (CVD) by increasing circulating levels of inflammation and haemostasis biomarkers. We investigated the associations of pericardial fat with inflammation and haemostasis biomarkers, as well as incident CVD events, and whether there are any ethnic differences in these associations.

Methods: We analysed results from 6415 participants from the Multi-Ethnic Study of Atherosclerosis who had measurements of pericardial fat volume and circulating levels of C-reactive protein, fibrinogen, interleukin (IL)-6, factor VIII, D-dimer and plasmin-antiplasmin complex (PAP), and had a mean follow-up period of 9.5 years. Incident CVD event was defined as any adjudicated CVD event.

Results: After adjusting for confounding factors, pericardial fat volume was positively associated with natural log (ln) of IL-6 levels, but inversely associated with ln D-dimer and ln PAP levels ($\beta=0.067$, -0.032 , and -0.105 respectively, all $P<0.05$). Although a larger pericardial fat volume was associated with a higher risk of incident CVD, the association was attenuated to borderline significance after adjusting for traditional cardiovascular risk factors ($P=0.050$). There was a borderline significant ethnicity interaction ($P=0.080$), whereby the association between pericardial fat volume and incident CVD was significant in Hispanic Americans, even after further adjusting for biomarkers of inflammation and haemostasis (hazard ratio=1.31 per standard deviation increase; 95% confidence interval, 1.09-1.57; $P=0.004$).

Conclusion: Pericardial fat was associated with several inflammation and haemostasis biomarkers. The association of pericardial fat with incident CVD events was independent of these biomarkers only among Hispanic Americans.

The relationship between insulin resistance and vascular calcification in coronary arteries, and the thoracic and abdominal aorta: the Multi-Ethnic Study of Atherosclerosis

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Objective: Insulin resistance may be related to vascular calcification as both are associated with abdominal obesity. We investigated the association of insulin resistance with abdominal aortic calcium (AAC), coronary artery calcium (CAC) and thoracic aortic calcium (TAC), and whether it differs according to different levels of subcutaneous fat area (SFA) and visceral fat area (VFA) in a cross-sectional study.

Methods: We investigated 1632 participants without diabetes from the Multi-Ethnic Study of Atherosclerosis with valid data on homeostasis model assessment index (HOMA-IR), AAC, CAC, and TAC. Adipocytokines, SFA, and VFA were also determined.

Results: HOMA-IR was associated with the presence of CAC, but not AAC and TAC, and the association remained significant after adjusting for traditional risk factors, adipocytokines, abdominal muscle mass, SFA, and VFA (prevalence ratio=1.04 per one interquartile range increase; P=0.01). As the strength of the association of HOMA-IR with vascular calcification may differ by abdominal fat composition, subgroup analysis was performed among participants with different tertiles of SFA and VFA. Significant interactions between HOMA-IR with SFA and VFA separately were observed for the presence of TAC, but not AAC and CAC, even after adjusting for confounding factors. The association of HOMA-IR with TAC tended to be stronger in participants with more SFA and VFA.

Conclusions: Atherosclerotic calcification, especially in the coronary arteries, is related to insulin resistance. Further studies are needed to delineate the mechanisms by which visceral obesity can lead to vascular calcification.

The relationship between total bilirubin levels and total mortality in older adults: the United States National Health and Nutrition Examination Survey (NHANES) 1999-2004

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Objective: Due to its anti-oxidant and anti-inflammatory properties, bilirubin has been associated with reduced cardiovascular risk. A recent study demonstrated an L-shaped association of pre-treatment total bilirubin levels with total mortality in a statin-treated cohort. We therefore investigated the association of total bilirubin levels with total mortality in a nationally representative sample of older adults from the general population.

Methods: A total of 4303 participants aged ≥ 60 years from the United States National Health and Nutrition Examination Survey 1999-2004 with mortality data followed up through 31 December 2006 were included in this analysis, with a mean follow-up period of 4.5 years.

Results: Participants with total bilirubin levels of 0.1-0.4 mg/dL had the highest mortality rate (19.8%). Compared with participants with total bilirubin levels of 0.5-0.7 mg/dL and in a multivariable regression model, a lower total bilirubin level of 0.1-0.4 mg/dL was associated with higher risk of total mortality (hazard ratio [HR]=1.36; 95% confidence interval [CI], 1.07-1.72; P=0.012), while higher levels (≥ 0.8 mg/dL) also tended to be associated with higher risk of total mortality, but this did not reach statistical significance (HR=1.24; 95% CI, 0.98-1.56; P=0.072).

Conclusion: In this nationally representative sample of older adults, the association of total bilirubin levels with total mortality was the highest among those with a level between 0.1 and 0.4 mg/dL. Further studies are needed to investigate whether higher total bilirubin levels could be associated with a higher mortality risk, compared to a level of 0.5-0.7 mg/dL.

The relationship of fibroblast growth factor 21 with cardiovascular outcome events in the Fenofibrate Intervention and Event Lowering in Diabetes Study

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Objective: Circulating fibroblast growth factor 21 (FGF21) levels are often elevated in obesity, dyslipidaemia, and type 2 diabetes. This study investigated the relationship of FGF21 levels with cardiovascular events in patients with type 2 diabetes.

Methods: Plasma FGF21 levels were measured at baseline in 9697 study participants with type 2 diabetes from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. We assessed the association of FGF21 levels with incidence of different cardiovascular outcomes over 5 years. The outcomes included total cardiovascular disease (CVD) events, coronary heart disease events, total stroke, CVD mortality, coronary and carotid revascularisation, and hospitalisation for angina pectoris.

Results: Higher baseline FGF21 levels were associated with higher risks of all cardiovascular outcome events after adjusting for the study treatment allocation (all $P < 0.01$). The associations remained significant for total CVD events, and coronary and carotid revascularisation after further adjusting for confounding factors with hazard ratio (95% confidence interval) being 1.28 (1.10, 1.50) and 1.26 (1.01, 1.56) respectively, for the highest tertile compared to the lowest tertile (overall effect $P = 0.002$ and 0.007 , respectively). The addition of FGF21 levels to a model including established CVD risk factors predicting total CVD led to a non-significant increase in the C-statistic, but resulted in significant integrated discrimination improvement and net reclassification improvement.

Conclusions: Higher baseline plasma FGF21 levels were associated with a higher risk of cardiovascular events in patients with type 2 diabetes.

Adipocyte fatty acid-binding protein deficiency prevents obesity-induced nephropathy in mice

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Introduction: Although our previous clinical evidences have shown that circulating levels of adipocyte fatty acid-binding protein (A-FABP) are positively related to the development of nephropathy, its potential role is unknown. The present study investigated the role of A-FABP in the pathogenesis of renal injuries associated with obesity.

Methods: A-FABP knockout (KO) mice and their wild-type littermates were either fed with high-fat-high-cholesterol (HFHC) diet or standard chow for 16 weeks. We collected renal tissues and analysed parameters through immunohistological staining, Western blot, and quantitative reverse-transcription polymerase chain reaction methods.

Results: Renal A-FABP expression was increased upon HFHC diet feeding. Pharmacological inhibition of A-FABP using a selective A-FABP inhibitor BMS309403 significantly attenuated HFHC diet-induced renal structural disorders. A-FABP deficiency decreased HFHC diet-induced up-regulation of plasma creatinine, urea micro-albumin, and urea protein. Furthermore, A-FABP KO mice showed alleviated HFHC diet-induced metabolic dysfunction and renal structural disorders and were accompanied by reduced macrophage infiltration, genes expression of inflammatory cytokines, and oxidative factors in kidney. As a key lipid transporter, A-FABP deficiency significantly reduced renal lipids uptake, HFHC diet-induced lipid deposits, and lipogenic gene expression.

Conclusion: Our current findings demonstrated that A-FABP deficiency protects mice from obesity-induced nephropathy at least partially through blocking the lipid transportation into the renal tissues.

Arsenic trioxide downregulates NPM-ALK in ALK-positive anaplastic large cell lymphoma

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Introduction: Anaplastic large cell lymphoma (ALCL) is an aggressive peripheral T cell lymphoma. ALK+ ALCL is a subtype of ALCL, defined by the presence of translocations involving anaplastic lymphoma kinase (ALK). ALK, a receptor tyrosine kinase which is silent or barely expressed in normal T cells, is translocated to N-terminal region of nucleophosmin (NPM1) with its full kinase domain and is subsequently highly expressed in 40% to 60% of ALCL. The standard first-line treatment for ALCL is CHOP-based chemotherapy. Although ALK+ ALCL may respond initially to chemotherapy, relapsed or refractory cases are frequently seen. In this study, arsenic trioxide (As₂O₃, ATO), a multi-target anti-cancer drug, was demonstrated to be an ALK suppressor that inhibits proliferation of ALCL in vitro.

Methods: We treated ALK+ ALCL cell lines or NPM-ALK-overexpressed 293T cells with As₂O₃ and examined its effects on NPM-ALK mRNA expression by semi-quantitative reverse-transcription polymerase chain reaction and protein expression by Western immunoblotting. The downstream signalling pathway of NPM-ALK, phosphorylation of STAT3 on Tyr705, was also examined. The effect of As₂O₃ on ALCL cell growth and cell death was examined with Annexin V apoptosis detection kit, cell cycle analysis, and cell proliferation assay.

Results: Our data showed that As₂O₃ downregulated NPM-ALK at the protein level without affecting the transcription in both ALK+ ALCL cell lines and NPM-ALK-overexpressed 293T cells. It also inhibited the phosphorylation of STAT3 in all ALK+ ALCL cell lines. Furthermore, As₂O₃ induced apoptotic cell death, cell cycle arrest at G2/M phase, and growth inhibition in ALK+ but not in ALK- ALCL cell lines.

Conclusion: Our data clearly demonstrated that ATO downregulates NPM-ALK at the protein level, thereby inhibits cell proliferation and induces cell death in ALK-positive ALCL. As₂O₃ may therefore represent a potential therapeutic agent for the treatment of ALK-positive ALCL.

Diffusion kurtosis imaging in detecting occult brain damage in multiple sclerosis and neuromyelitis optica

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Introduction: Multiple sclerosis (MS) and neuromyelitis optica (NMO) are central nervous system inflammatory demyelinating disorders. Conventional magnetic resonance imaging (cMRI) is useful to detect lesions in MS and NMO, but pathological studies show that histological abnormalities can occur in normal-appearing white matter (NAWM) and normal-appearing grey matter (NAGM) on cMRI. Diffusion kurtosis imaging (DKI) is a new MRI technique which estimates conventional diffusion tensor imaging metrics more accurately with additional metrics quantifying degree of non-Gaussian water diffusion. We studied whether DKI can detect lesions in NAWM and NAGM on cMRI in MS and NMO patients.

Methods: Chinese MS and NMO patients followed up in neurology clinic of Queen Mary Hospital had cerebral cMRI and DKI performed in MRI unit of the University of Hong Kong on a Philips 3T MRU Achieva scanner with a body coil excitation and an 8-channel SENSE head coil for reception. Values were compared to that of healthy volunteers (HV).

Results: Overall, 13 NMO (11 females; mean age, 44.5 ± 13.0 years; mean Expanded Disability Status Scale [EDSS] score, 3.0 ± 1.7) and 18 MS patients (12 females; mean age, 42.1 ± 10.1 years; mean EDSS, 2.9 ± 1.7) were recruited in this pilot study. Volumetric analysis showed no differences in volume of white matter (WM), grey matter (GM), peripheral GM, and total brain parenchyma in MS, NMO, and HV. For NAWM, fractional anisotropy (FA) and kurtosis metrics were decreased in frontal, parietal, and temporal lobes of MS patients compared to that of HV and NMO. For NAGM, FA and K_{||} were reduced while diffusivity indices were increased in MS, and FA was reduced but diffusivities were enhanced in frontal and temporal lobes of NMO. For subcortical NAGM, increased mean diffusivity (MD) and λ_⊥ with decreased K_{||} were observed in thalamus of NMO, and increased FA, λ_{||}, MK and K_⊥ with no changes in λ_⊥ and K_{||} were observed in caudate nucleus and putamen of MS. For thalamus, all diffusion and kurtosis metrics were significantly enlarged except K_{||} in MS and NMO.

Conclusion: DKI facilitates detection of lesions of NAWM and NAGM in MS and NMO.

Validation of AD-CSF-Index in Chinese patients with Alzheimer's disease and non-demented controls

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Introduction: Cerebrospinal fluid (CSF) biomarkers of Alzheimer's disease (AD) especially A β 42/t-tau and A β 42/p-tau showed high diagnostic sensitivities and specificities. However, significant interassay and interlaboratory variabilities hinder the widespread clinical applications of CSF biomarkers. The objective of this study was to validate the diagnostic accuracy of AD-CSF-Index in our local Chinese AD patients as compared to non-demented controls.

Methods: A total of 36 subjects, which included 24 AD patients and 12 non-demented controls, were recruited from the Memory Clinic, Queen Mary Hospital.

Results: Chinese AD patients showed higher mean AD-CSF-indices than non-demented controls (AD=1.25-1.3, normal=0.62-0.63; $P < 0.001$). The AD-CSF-index has a high sensitivity and specificity 86.4%-90.9% and 83.3%, respectively in differentiating AD patients from non-demented control. After selecting the "pure AD" and "pure normal" subjects, AD-CSF-indices have a high sensitivity and specificity 94.1% and 90%, respectively in differentiating AD patients from non-demented control.

Conclusion: AD-CSF-Indices showed high sensitivity and specificity in discriminating AD patients from non-demented control in Chinese. Our AD-CSF-Indices cut-offs were very similar to other reported western studies cut-offs suggesting their potential usefulness in harmonising CSF biomarkers interpretation between centres.

Acknowledgement

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Comparisons of clinical symptoms in biomarkers-confirmed Alzheimer's disease, dementia of Lewy bodies and frontotemporal dementia patients in a local Memory Clinic

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Introduction: There is no previous Chinese study on differentiating clinical symptoms among biomarkers-confirmed Alzheimer's disease (AD), dementia of Lewy bodies (DLB), and frontotemporal dementia (FTD). The objective of this study was to compare the cognitive, behavioural, and neuropsychiatric symptoms (BPSD) in biomarkers-confirmed AD, DLB, and FTD patients.

Methods: We recruited 30 patients (14 AD, 7 DLB, 9 FTD) presented to Memory Clinic in Queen Mary Hospital between 1 January 2007 and 31 December 2013. Subjects were matched by dementia severity and duration of symptoms before presentation. AD was diagnosed according to NINCDS-ADRDA criteria with CSF biomarkers (tau, p-tau, and A β 42 by Innostest ELISA) fulfilling locally determined cut-off values for AD. DLB was diagnosed by McKeith diagnostic criteria. Behavioural variant of FTD was diagnosed by revised diagnostic criteria proposed by the International bvFTD Criteria Consortium and language variant FTD was diagnosed by latest published criteria. In addition, patients with DLB and FTD had typical imaging features on SPECT/¹⁸F-DG-PET +/- PIB supporting their diagnoses. Data on patient characteristics including demographics, presenting clinical features, Mini-Mental State Examination, clinical dementia ratings, and neuropsychiatry inventory scores were collected.

Results: There were no differences on age, education level, dementia severity, and duration of symptoms before presentation among the three subgroups of patients. All patients had amnesia symptoms (P =not significant). Apraxia was the most common in AD. Of the patients, 83% were affected by BPSD symptoms. However, behavioural disinhibition and decline in executive function were most common in FTD patients. Recurrent hallucinations, fluctuation of consciousness, parkinsonism, and rapid eye movement sleep disturbance symptoms were most common in DLB.

Conclusion: Memory impairment and apathy are not useful discriminative symptoms in diagnosing AD, DLB, and FTD. Apraxia favours AD. Hallucinations, particularly well-formed visual hallucinations, favour DLB. Overall, BPSD symptoms are common among the three groups of dementia patients.

Prevalence of cognitive impairment among peritoneal dialysis patients, impact on peritonitis, and role of assisted dialysis

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Objective: Chronic renal failure and ageing are suggested as risk factors for cognitive impairment (CI). We studied the prevalence of CI among peritoneal dialysis (PD) patients using Montreal Cognitive Assessment (MOCA), its impact on PD-related peritonitis in the first year, and the potential role of assisted PD.

Methods: A total of 114 patients were newly started on PD between February 2011 and July 2013. MOCA was performed in the absence of acute illness. Data on patient characteristics including demographics, comorbidities, blood parameters, dialysis adequacy, presence of helpers, medications, and the number of PD-related infections were collected.

Results: The mean age of studied patients was 59±15.0 years and 47% were female. The prevalence of CI was 28.9%. Patients older than 65 years (odds ratio [OR]=4.88; confidence interval, 1.79-13.28; P=0.002) and with an education of primary level or below (OR=4.08; confidence interval, 1.30-12.81; P=0.016) were independent risk factors for CI in multivariate analysis. Patients with PD-related peritonitis were significantly older (P<0.001) and more likely to have CI as defined by MOCA (P=0.035). Although after adjustment of age, CI was not a significant independent risk factor for PD-related peritonitis among self-care PD patients (OR=2.20; confidence interval, 0.65-7.44; P=0.20). When we compared patients with MOCA-defined CI receiving self-care and assisted PD, there were no statistically significant differences between the two groups in terms of age, MOCA scores, or comorbidities. There were also no statistically significant differences in 1-year outcome of PD-related peritonitis rates or exit-site infections.

Conclusion: CI is common among local PD patients. Even with CI, peritonitis rate in self-care PD with adequate training is similar to non-CI patients on self-care PD and CI patients on assisted PD. In the future, we still need to conduct a study with a larger sample size of PD patients with CI in order to study whether CI is a risk factor for PD peritonitis given that 44% of CI patients on self-care PD developed peritonitis within the first year compared with 22% in CI patients on assisted PD.

The efficacy of a 532-nm and 1064-nm picoseconds laser for the treatment of benign pigmented lesions in Chinese

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Introduction: Laser treatment of benign pigmented lesions have been studied widely. Q-switched laser for pigmented lesions in Asians reported a 25% post-inflammatory hyperpigmentation (PIH) risk. Long-pulsed Nd:YAG was reported to have a lower PIH risk. Recently, picosecond lasers were introduced. The objective of this study was to assess the safety and efficacy of a new dual-wavelength picosecond laser for the treatment of benign pigmented lesions.

Methods: Thirty patients with benign pigmented lesions were recruited. Three to nine treatment sessions at 2 to 6 weeks' interval were carried out depending on the type of lesion and response to treatment. The treatment wavelength and pulse energy were selected by the treating physician based on the lesion and subjects' phototype. The discomfort level, adverse effects, clearance rate, and standardised photographs were recorded at all visits. The final follow-up visits took place at 4, 8, and 12 weeks after the last treatment session. Standardised photographs were assessed by two independent blinded physicians.

Results: At this point of time, 12 subjects with 14 types of benign pigmented lesions have started treatment. The average pain score (Visual Analogue Scale score) over 38 treatment sessions was 4.0. The average satisfaction level recorded at the most recent visit was 75%. Subjects with melasma had good response at the sixth treatment onwards. Of the five subjects with freckles and lentigines, three had good-to-excellent response after one treatment. Those with cafe au lait reported good-to-excellent response after two treatments. One subject with Hori's macule and lentigines experienced two episodes of blistering which healed without scarring. Otherwise there were no serious adverse effects.

Conclusion: The study is ongoing and final data are yet to be generated. As of now the result is promising.

Acknowledgement

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The efficacy of a second-generation focused ultrasound combined with radiofrequency for body contouring in Chinese

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Introduction: Several modalities for body contouring have been available commercially in the past few years, one of which is focused ultrasound. Fat cell destruction is achieved by ultrasound-induced mechanical effects. The second-generation device aims to be safer with improved efficacy. The objective of our study was to assess the efficacy of this second-generation focused ultrasound device in Chinese.

Methods: Twenty subjects were recruited for the treatment of abdominal fat. More than 1.5-cm fat thickness at the target area was required. Informed consent was obtained after going through the procedure and list of contra-indications with the subject. All subjects received three successive treatments biweekly. Additional six visits were required for follow-up; the last visit was 3 months after last treatment. A combined treatment included radiofrequency treatment in stacking mode followed by ultrasound treatment immediately after. Caliper reading, abdominal circumference, and standardised photographs were taken with the Vectra® system at all visits. Circumferential measurement of right thigh served as control. A transparent template for each subject was used to ensure the same area was treated at each session.

Results: Overall, 17 female subjects completed the study. Abdominal circumference showed statistically significant improvement at 2 weeks' post second treatment and all subsequent follow-ups. Caliper readings were statistically significant at 2 weeks post second treatment till 1-month follow-up visit. The mean pain score reported was 2.3 on the visual analogue scale. Six incidents of wheal formation appeared immediately after treatment; all of which subsided spontaneously within several hours. Otherwise there were no adverse effects.

Conclusion: The second-generation focused ultrasound combined with radiofrequency treatment for body contouring showed statistically significant improvement. It was a comfortable procedure with no significant adverse effects.

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A retrospective study of a 755-nm picosecond laser for the treatment of benign pigmented lesions in Chinese

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Introduction: Photoaging in Chinese often presents with benign pigmented lesions. Q-switched laser for pigmented lesions in Asians reported a 25% post inflammatory hyperpigmentation (PIH) risk whilst long-pulsed Nd:YAG was reported to have a lower PIH risk. Recently, picosecond lasers of various wavelengths were introduced. The objective of this study was to assess the efficacy of a new 755-nm picosecond laser for the treatment of benign pigmented lesions retrospectively.

Methods: A list of patients who received 755-nm picosecond laser treatment at our centre was taken. Those who had any other laser or topical treatment during the period of 755-nm picosecond laser treatment were excluded from the study. The age, skin type, type of lesion, and number of treatments performed were recorded. The baseline and most recent standardised photographs were assessed by trained physicians for comparison. A score of 0-4 representing poor 0-24%, good 25-49%, excellent 75-95%, and complete 95%+ improvement was given.

Results: A total of 13 subjects were included. The number of treatment sessions received ranged from one to seven. The benign pigmented lesions consisted of nevus of ota, café au lait patches, lentigines, Becker's nevus, Hori's macules and nevus spilus. A case of nevus of ota achieved complete clearance after four treatments, and two other patients with nevus of ota had excellent clearance after three and four sessions. Patients with café au lait had fair-to-good clearance after one to seven sessions. One patient who had Hori's macules was the most resistant to treatment with fair response after eight treatments. Two patients developed hypopigmentation, a rate of 4.8% (2 out of 42 treatment sessions) and none had PIH.

Conclusion: The new 755-nm picosecond is effective for the treatment of benign pigmented lesions in Chinese especially for the clearance of nevus of ota. There is a small risk of hypopigmentation.

Functions of *idh1* and its mutation in the regulation of developmental haematopoiesis in zebrafish

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Introduction: Isocitrate dehydrogenase 1 mutation (IDH1-R132H) was recently identified in acute myeloid leukaemia (AML) with normal cytogenetics. The mutant enzyme is thought to convert alpha-ketoglutarate (alpha-KG) to the pathogenic 2-hydroxyglutarate (2-HG) that affects DNA methylation via inhibition of 10-11 translocation 2. However, the role of wild-type IDH1 in normal haematopoiesis and its relevance to AML is unknown.

Methods: Zebrafish *idh1* (*zidh1*) was knocked-down by anti-sense morpholino and targeted mutagenesis by transcription activator-like effector nuclease (TALEN) or overexpressed by injecting cDNA encoding IDH1 wild-type or disease-related mutation form. The haematopoietic phenotype was analysed by whole-mount in-situ hybridisation, real-time reverse transcription polymerase chain reaction, and quantitatively by flow cytometry in transgenic embryos as well as pharmacological treatment.

Results: We showed that both morpholino knockdown and TALEN-induced targeted mutagenesis of *zidh1* induced blockade in myeloid differentiation, as evident by an increase in myeloid progenitor (*pu.1*) and decrease in differentiated myeloid cells (*mpo*, *l-plastin* and *mpeg1*), and significantly reduced definitive hematopoiesis (*c-myb* and *rag1*). Morpholino knockdown of *zidh2* also induced a blockade in myeloid differentiation but definitive haematopoiesis was not affected. The haematopoietic phenotype of *zidh1* knockdown was not rescuable by *zidh2* mRNA, suggesting non-redundant functions. Over-expression of human IDH1-R132H or its zebrafish orthologue resulted in 2-HG elevation and expansion of myelopoiesis in zebrafish embryos. A human IDH1-R132H specific inhibitor (AGI-5198) significantly ameliorated both haematopoietic and 2-HG responses in human but not zebrafish IDH1 mutant expression.

Conclusion: *Zidh1* plays an important role in the regulation of myelopoiesis and definitive haematopoiesis. Expression of human IDH1-R132H and its zebrafish orthologue induced an increase in myelopoiesis and 2-HG.

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Adipocyte fatty acid binding protein is a potential regulator of browning in white adipose tissue

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Introduction: ‘Browning’ in white adipose tissue (WAT) can be induced by cold exposure to enhance adaptive thermogenesis. Increased WAT browning is shown to counteract obesity and metabolic diseases in rodents and humans. Adipocyte fatty acid-binding protein (A-FABP) is an adipokine that mainly expressed in adipocytes and macrophages. Previous study demonstrated that A-FABP is a regulator of adaptive thermogenesis in brown adipose tissue (BAT). Thyroid hormones, triiodothyronine (T3) and its prohormone, thyroxine (T4), also play a crucial role in adaptive thermogenesis. Here, we investigated the role of A-FABP in WAT browning and its underlying mechanism.

Methods: A-FABP knockout (KO) mice and their wild-type (WT) littermates after 4 weeks of standard chow or high-fat-high-cholesterol (HFHC) diet feeding were subjected to cold induction (4°C) for 24 hours. On the other hand, A-FABP KO mice were replenished with A-FABP recombinant protein or phosphate buffered saline (PBS) by osmotic pump for 14 days followed by cold exposure for 8 hours. Energy expenditure of the above mice was determined by Columbus Instruments Comprehensive Lab Animal Monitoring System (CLAMS). Histological analysis of BAT and WAT was performed by haematoxylin and eosin staining. The expression of thermogenic gene such as uncoupling protein 1 (UCP-1) and *Dio2* gene were determined by quantitative polymerase chain reaction and Western blot analysis.

Results: Circulating A-FABP and its expression in WAT of WT mice were significantly increased upon cold challenge, while A-FABP KO mice demonstrated a defective adaptive thermogenesis comparing to WT controls. Replenishment of A-FABP enhanced thermogenic ability of KO mice and was accompanied by ‘browning’ in WAT associating with increased multi-locular structure (beige cells) and UCP-1 expression. Notably, the expression of *Dio2*, the gene encoding type 2 deiodinase which is the enzyme responsible for intracellular conversion of T4 to activated T3, was significantly increased.

Conclusion: A-FABP increases energy expenditure by inducing ‘browning’ in subcutaneous adipose tissue which may through enhancing intracellular T3 level via activation of *Dio2* signal pathway.

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Transfusion support is an essential part of haematopoietic stem cell transplant (HSCT). Platelet components for transfusion are stored at room temperature as cold temperatures induce aggregation of von Willebrand factor receptors and subsequent platelet elimination by liver macrophages. However, storage at room temperature increases the risk of bacterial contamination (estimated to be 1/2000 to 1/3000 platelet units).

The amotosalen / UVA-based INTERCEPT Blood System (IBS; Cerus Corporation, Concord CA, US) is one pathogen inactivation technology which inactivates pathogen through a photochemical reaction preventing DNA replication and RNA transcription. I-PLT (INTERCEPT-treated Platelet) has the further advantage of lymphocyte inactivation, obviating the need for gamma-irradiation for transfusion-associated graft-versus-host disease (TA-GvHD) prophylaxis in HSCT recipients. IBS has received national registration in France (Afssaps), Germany (Paul Ehrlich Institute), and Switzerland (Swissmedic) as well as in other countries. Here we conduct a prospective study to evaluate the therapeutic efficacy and safety of I-PLT in Hong Kong Chinese HSCT recipients.

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Introduction: Drug-induced agranulocytosis is a rare but serious adverse drug reaction. There are, however, limited studies on the epidemiology of drug-induced agranulocytosis in the Chinese population.

Methods: We performed a descriptive analysis on drug-induced agranulocytosis in Hong Kong. Data were obtained from Hong Kong Clinical Data Analysis and Reporting System database. Patients with agranulocytosis (ICD-9 code: 288.0) during 2002 to 2012 were investigated. Laboratory data and prescription records were reviewed to identify drug-induced cases.

Results: A total of 215 cases were identified from the database. The incidence rate was estimated to be 2.58 per million people per year. The mean age was 53±20.9 years and male-to-female ratio was 0.71:1. Case fatality rate was 7.4% and all the deaths were aged over 60 years. Anti-thyroid drugs (33%) was the most common group of causative drugs, in which 62% were derived from carbimazole. Antibiotics were the second most common in 32.6% of patients with penicillin (34.3%) being the most implicated drug. Anticonvulsants were responsible in 9.3% of cases, in which phenytoin (65%) was more common than other anticonvulsants. Other causative drugs include antipsychotics (clozapine), gout suppressants (allopurinol, colchicine), antiplatelet agent (clopidogrel), and iron-chelating agent (deferiprone).

Conclusion: This is the first epidemiological study in Southern Chinese population. Results were comparable to studies in other populations.

Dissecting the physiological role of the novel lupus-associated C-type lectin-like protein CLEC16A

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Introduction: The *CLEC16A* locus has been identified as a susceptibility gene for multiple autoimmune diseases, including type-1 diabetes and systemic lupus erythematosus (SLE) in genome-wide association studies. *CLEC16A* encodes a novel C-type lectin-like protein, by virtue of a predicted C-type lectin-like domain. The *Drosophila* ortholog of CLEC16A, Ema, has been reported to regulate endosomal protein trafficking and the autophagic process, while the roles of mammalian CLEC16A remain unclear. This study was undertaken to investigate the function(s) of CLEC16A in mammalian cells.

Methods: CLEC16A was overexpressed in two human epithelial cell lines. The expression pattern was evaluated using flow cytometric analysis and immunofluorescence microscopy (IF) with multiple organelle markers. To evaluate a role for autophagy, CLEC16A-overexpressing cells were starved or stimulated with rapamycin, which are commonly used protocols to induce autophagy. The level of autophagic activity was measured by LC3-immunoblotting.

Results: CLEC16A was found to be an intracellular protein with a punctate cytoplasmic expression. By IF, it was not found to reside in endoplasmic reticulum, early endosomes, lysosomes, or autophagosomes. However, starved cells and rapamycin-treated cells exhibited a down-regulated autophagic response in overexpression of CLEC16A.

Conclusions: CLEC16A appeared to exert an inhibitory role on the autophagic pathway in mammalian cells. Although in steady state the place of residence of CLEC16A was not identified, CLEC16A puncta interestingly displayed a translocation within the cytoplasm upon starvation. As such, which organelle CLEC16A would locate in when autophagy is induced should be investigated. The molecular mechanism through which CLEC16A regulates autophagy should be delineated. Since autophagy has important implications in the regulation of immune pathways, such knowledge would be instrumental in linking CLEC16A with the pathogenesis of SLE.

A local study of the bacteriology of urine cultures from patients with non-traumatic spinal cord injury

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Background: Spinal cord injury (SCI) is very commonly associated with bacteriuria and urinary tract infections (UTI). There are no local studies concerning the sensitivity pattern of microorganisms isolated from urine from these group of patients, which is important in deciding antibiotic regimen in case of UTI.

Objectives: To study the culture and antibiotic sensitivity pattern of microorganisms isolated from urine cultures from patients suffering from non-traumatic SCI in a local medical rehabilitation centre.

Methods: All the in-patient urine cultures of patients with clinical and/or radiological evidence of spinal cord injury admitted to MacLehose Medical Rehabilitation Centre during a 6-year period were analysed.

Results: Overall, 19 patients fulfilled the inclusion criteria. The mean age of the subjects was 51.6 years and the mean time from the onset of the spinal lesion was 4.06 months. The commonest method for bladder management was intermittent catheterization, which was found in 52.6% of the patients. From them a total of 180 urine culture specimens were collected; 69.4% (125 samples) had significant growth of microorganisms. The commonest organisms isolated were *Escherichia coli* (43.3%), *Klebsiella* species (17.3%), *Enterococcus* species (15.0%), and *Pseudomonas aeruginosa* (7.9%). Sensitivities of the *E coli* isolates to ampicillin, co-trimoxazole and nalidixic acid were low (all below 40%) and 20% of the isolates were extended spectrum beta lactamase-producing strains. Sensitivities to cefuroxime and amoxicillin-clavulanate were maintained above 75%. The *Klebsiella* and *Pseudomonas* isolates were generally sensitive to most of the commonly used antibiotics.

Conclusion: The culture and sensitivity pattern were quite in line with nosocomial UTI in general.

The role of hypothalamic APPL2 in regulation of energy metabolism

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Introduction: The adaptor protein APPL2 has been identified as a regulator of glucose metabolism in skeletal muscle, however, its physiological function in other tissues still remains obscure. To investigate the physiological role of APPL2 in pancreatic beta cells and hypothalamus, we generated tissues-specific knockout (KO) mouse models using the Cre-LoxP approach.

Methods: APPL2^{fllox/fllox} mice were crossed with transgenic mice expressing Cre recombinase under the control of rat insulin promoter (RIP) to generate RIP-APPL2 KO mice, which has diminished APPL2 expression in both hypothalamus and β -cells. Adeno-associated virus (AAV) expressing Cre recombinase or green fluorescent protein was introduced by local injection into hypothalamus of APPL2^{fllox/fllox} mice. Basic metabolic and glucose parameters of the above mice were continuously monitored for 24 weeks.

Results: Apart from glucose intolerance, RIP-APPL2 KO mice displayed increased adiposity accompanied by a dramatic reduction of energy expenditure, despite similar body weight, food intake, and locomotor activity. Upon chronic cold challenge, induction of beige cell within subcutaneous white adipose tissue (sWAT) was almost absent in RIP-APPL2 KO mice whereas no obvious change appeared in their brown adipose tissue (BAT). Administration of beta3-adrenergic receptor agonist reversed defective adaptive thermogenesis in sWAT of RIP-APPL2 KO mice, indicating the defect might be due to dysregulated hypothalamic function. In addition, absence of beige cell recruitment in sWAT of RIP-APPL2 KO mice was associated with selectively decreased sympathetic nervous system activity. The role of hypothalamic APPL2 in regulating thermogenesis in sWAT was further supported by data showing AAV-mediated deletion of APPL2 in hypothalamus caused cold intolerance, reduced energy expenditure, and increased adiposity in mice.

Conclusion: Hypothalamic APPL2 may differentially regulate sympathetic outflow to sWAT and BAT, thereby controlling thermogenic responses in adipose tissues and maintaining energy homeostasis.

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The role of visfatin in intermittent hypoxia-induced inflammation and endothelial dysfunction in ea.hy926 cells

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Introduction: Intermittent hypoxia (IH), a hallmark feature in obstructive sleep apnoea (OSA) may be the main culprit underlying endothelial dysfunction. Visfatin is a multifaceted adipokine, whose extracellular form can exert various effects on the vascular endothelium including inflammation, proliferation, etc. However, little is known about the role of visfatin in inflammation and endothelial dysfunction induced by IH.

Methods: IH exposure was performed in the hypoxic chamber (O₂ levels: 1% for 10 mins and 21% for 5 mins in one complete cycle and 5% CO₂). Intermittent normoxia (IN; 21% O₂ and 5% CO₂) exposure was carried out simultaneously in control cell cultures. Endothelial EA.hy926 cells were exposed to IN or IH for 96 cycles with two concentrations of visfatin (10 ng/mL and 100 ng/mL). Supernatants were collected after treatment to measure the levels of inflammation-associated markers interleukin-8 (IL-8), macrophage chemoattractant protein-1 (MCP-1), and transforming growth factor beta 1 (TGF- β 1) using enzyme-linked immunosorbent assay. Cellular proteins were extracted to determine the expression of nitric oxide synthase (eNOS) and its activity in terms of phosphorylation at ser1177 (p-eNOS [Ser1177]) using Western blot.

Results: IH exposure induced elevation of IL-8 and MCP-1 levels and reduction of TGF- β 1 level (P<0.05). Visfatin attenuated IH-induced elevation of IL-8 and MCP-1 levels and reduction of TGF- β 1 level in a dose-dependent manner. Western blot analysis revealed that IH exposure significantly suppressed p-eNOS expression but not eNOS expression in EA.hy926 cells. In the presence of visfatin, no differences were found in the basal expression of p-eNOS and eNOS or in IH-induced suppression of p-eNOS.

Conclusion: These results provided evidence that visfatin might play a pivotal role in IH-induced endothelial inflammation but not in reversing endothelial dysfunction in OSA.

Profound reduction of HBV covalently closed circular DNA with long-term nucleoside/tide analogue therapy

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Background: Long-term nucleoside/tide analogue (NA) treatment suppresses serum hepatitis B virus (HBV) DNA to undetectable levels in a majority of patients. We investigated the effect of long-term NA on the reduction of covalently closed circular DNA (cccDNA), intrahepatic HBV DNA (ihHBV-DNA), and pregenomic RNA (pgRNA).

Methods: We recruited 43 patients (median age, 43.5; range, 24.3-63.2 years) who had been on continuous long-term (5-12 years) NA. All patients had three liver biopsies: at baseline, after 1 year of treatment, and at the last follow-up. Levels of serum HBV DNA and HBsAg, ihHBV-DNA, cccDNA, and pgRNA were measured.

Results: The median duration of treatment was 10.4 years (range, 6.0-11.9 years). Histology of the third biopsy showed 0-1 confluent necrosis, piecemeal necrosis, apoptosis, and portal inflammation in the majority of patients. Overall, 12 (28%) patients had 0-1% staining for HBsAg. All but one (2.3%) patient stained negative for HBcAg. At the time of the last biopsies, 38 (88%) patients had undetectable serum HBV DNA (<20 IU/mL), all but one patient still had detectable HBsAg (median, 2.88 logIU/mL), all had detectable ihHBV-DNA (median, 0.35 copies/cell), and 21 (49%) patients had undetectable cccDNA. HBV pgRNA was detectable at a very low level (median, 0.021 copies/cell; 40% undetectable). The median percentage reductions of HBsAg, ihHBV-DNA, and cccDNA at last biopsies were 71.46%, 99.84%, and 99.89%, respectively.

Conclusions: Long-term NA treatment significantly reduced cccDNA and ihDNA. 49% of patients had undetectable cccDNA, although small amounts of ihHBV-DNA were still detectable in all patients; 40% had undetectable pgRNA.

Utilisation of medications to lower blood pressure, glucose, and lipids among people with type 2 diabetes in the National Health and Nutrition Examination Survey 1999-2010

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Objective: Changes in drug treatment of diabetes in the United States were studied using data from the National Health and Nutrition Examination Survey 1999-2010.

Methods: Data on 3094 participants aged ≥ 20 years with diagnosed type 2 diabetes were analysed. The use of medications for lowering blood glucose, blood pressure (BP), and lipids in the past month was assessed by questionnaire. Data from two survey cycles were combined together to produce estimates for each 4-year period.

Results: Usage of all three types of medications increased significantly from 1999-2002 to 2007-2010 ($P < 0.01$). Metformin was increasingly used from 34.8% to 53.8% during this period ($P < 0.001$), which was the most common medication for diabetes in 2003-2010, and half of the subjects taking metformin could achieve glycated haemoglobin of $< 7.0\%$ in 2007-2010. Dipeptidyl peptidase-4 (DPP-4) inhibitors, approved in 2007, were used by 7.4% of the participants in 2007-2010. Usage of angiotensin receptor blockers (ARB) and beta-blockers increased from 7.4% to 21.4%, and from 15.3% to 31.8%, respectively, across the 12-year period (both $P < 0.001$). 52.2% of subjects took statins by 2007-2010 ($P < 0.001$).

Conclusion: There were significant increases in the use of glucose, BP, and lipid-lowering medications during 1999-2010, especially in the use of metformin, ARB and beta-blockers. The increased BP drug prescription could lead to improved BP control. Metformin is the recommended first-line drug for diabetes, while DPP-4 inhibitors began to be used after their introduction. Although statins were widely used, about half of the participants did not take them.

Alternatively activated dendritic cells derived from systemic lupus erythematosus patients have tolerogenic phenotype and function

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Background: Tolerogenic dendritic cells (DCs) are potential cell-based therapy in autoimmune diseases.

Methods: In this study, we generated alternatively activated DCs (aaDCs) by treating monocyte-derived DCs from patients with systemic lupus erythematosus (SLE) and healthy subjects with combination of 1,25 dihydroxyvitamin D(3) (vitD3) and dexamethasone followed by lipopolysaccharide-induced maturation.

Results: Lupus aaDCs were found to acquire semi-mature phenotype that remained maturation-resistant to immunostimulants. They produced low level of interleukin-12 (IL-12) but high level of IL-10. They had attenuated allostimulatory effects on T cell activation and proliferation comparable to normal aaDCs and demonstrated differential immunomodulatory effects on naïve and memory T cells. These aaDCs were capable of inducing IL-10 producing regulatory T effectors from naïve T cells whereas they modulated cytokine profile with suppressed production of interferon- γ and IL-17 by co-cultured memory T cells with attenuated proliferation.

Conclusions: These aaDCs were shown to be superior than those generated using vitD3 alone in lupus patients.

Efficacy and tolerability of long-term tacrolimus treatment in lupus nephritis

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Introduction: Tacrolimus (TAC) is an emerging treatment for lupus nephritis (LN), but long-term data of this therapy are lacking.

Methods: We retrospectively reviewed 27 LN patients who received TAC treatment for 46.9 \pm 37.9 months.

Results: In 17 patients with class III/IV or V LN and persistent proteinuria of >2 g/day despite induction immunosuppression, add-on TAC treatment resulted in response rates of 66.7% and 80.0%, respectively. In 10 patients with nephrotic syndrome due to class V LN, prednisolone and TAC as initial treatment was associated with response rates of 60.0% and 90.0% after 12 and 24 months, respectively. The overall proteinuria dropped from 3.6 \pm 2.6 g/d to 1.0 \pm 1.1 g/d (P<0.05). Four patients developed end-stage kidney disease, with 3-, 5-, and 8-year renal survival rates of 93%, 83%, and 83%, respectively. In the remaining patients, serum creatinine and estimated glomerular filtration rate remained stable after 36 months. One patient with pre-existing chronic renal failure developed TAC nephrotoxicity. Four renal relapsed occurred, and all were associated with low TAC blood levels. Six (20.1%) patients had deterioration of hypertension and one (3.4%) patient had new-onset diabetes mellitus. Six (20.1%) patients had infections that required hospitalisation. Two patients succumbed, and was due to pneumonia and breast cancer respectively.

Conclusion: The results suggest efficacy of TAC in LN, especially in reducing proteinuria. Its role as long-term maintenance agent remains to be examined.

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Introduction: Entecavir (ETV) is efficacious for both treatment-naïve and lamivudine (LAM)-resistant hepatitis B virus (HBV) infection. Data on ETV treatment in renal transplant recipients (RTR) is limited.

Methods: The efficacy and tolerability of ETV in HBsAg+ RTR at Queen Mary Hospital during 2005 to 2013 were retrospectively reviewed.

Results: Overall, 21 RTR (10 treatment-naïve, 11 with LAM resistance) received ETV treatment for 34.7 ± 22.9 months (range, 6-75 months). ETV therapy led to a drop of HBV DNA titre compared to baseline and was more remarkable in the treatment-naïve group (treatment-naïve: $P=0.028$, <0.001 and <0.001 ; LAM-resistant $P=0.273$, 0.180 , and 0.109 after 12, 24, and 36 months, respectively). 60%, 100%, and 100% of patients in treatment-naïve group, and 27%, 45%, and 45% in the LAM-resistant group had undetectable HBV DNA after 12, 24, and 36 months of treatment, respectively. Time-to-undetectable HBV DNA and time-to-alanine transaminase (ALT) normalisation were 15.7 ± 4.6 and 12.6 ± 3.7 months for treatment-naïve patients, and 24.5 ± 4.2 and 28.2 ± 3.5 months for those with LAM resistance, respectively. Two patients in the LAM-resistant group developed genotypic resistance to ETV after 20.0 ± 3.5 months with rise in ALT and HBV DNA in two patients with LAM resistance. ETV resistance did not occur in the treatment-naïve group. No allograft dysfunction, de-novo cirrhosis, or hepatocellular carcinoma was observed during follow-up.

Conclusion: ETV is efficacious for both treatment-naïve and LAM-resistant HBsAg+ RTR, although treatment effect is less prominent in the latter group. The tolerability profiles of ETV in both groups were favourable.

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Introduction: In-vitro data showed that immunoglobulin G (IgG) from lupus nephritis (LN) patients could bind to proximal renal tubular epithelial cells (PTEC), but the clinical relevance of such PTEC-binding IgG remains unclear.

Methods: Binding of IgG and subclasses to PTEC (expressed as OD index) was measured in 189 serial serum samples from 23 patients with Class III/IV±V LN (48 during renal flares, 141 during remission), and compared with 64 patients with non-lupus glomerular diseases (NLGD) and 23 healthy individuals.

Results: IgG PTEC-binding index was 0.34 ± 0.16 , 0.29 ± 0.16 , 0.62 ± 0.27 , and 0.83 ± 0.38 in serum samples from healthy controls, NLGD, LN patients during LLDA remission, and LN patients during nephritic flare, respectively ($P < 0.001$, LLDA vs renal flare; $P < 0.001$, healthy controls or NLGD vs LN during remission or renal flare). PTEC-binding index for IgG₁ was 0.09 ± 0.05 , 0.16 ± 0.12 , 0.44 ± 0.34 , and 0.71 ± 0.46 for the corresponding groups ($P < 0.001$, LLDA vs renal flare; $P < 0.001$, healthy controls or NLGD vs LN during LLDA or renal flare). Total IgG and IgG₁ PTEC-binding correlated with the level of anti-dsDNA antibodies ($r=0.34$ and 0.52 respectively, $P < 0.001$ for both), and inversely with C3 level ($r=-0.26$ and -0.50 respectively, $P=0.002$ and <0.001). Sensitivity/specificity of PTEC-binding index in detecting renal flares was 45.8%/80.1% respectively for total IgG (area under a receiving operating characteristic curve [ROC AUC]=0.630, $P=0.007$) and 87.5%/35.5% respectively for IgG₁ (ROC AUC=0.615, $P=0.018$). IgG₁ PTEC-binding index correlated with tubulo-interstitial inflammation score in renal biopsy from corresponding patients.

Conclusion: Total IgG and IgG₁ PTEC-binding index in serum of LN patients correlate with serological activity, and in combination could predict renal flares. The correlation between IgG₁ PTEC-binding and tubulo-interstitial inflammation suggests a potential pathogenetic significance.

Interaction between proximal tubular epithelial cells and T lymphocytes: the role in tubulointerstitial inflammation in lupus nephritis

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Introduction: Renal proximal tubular epithelial cells (PTEC) and T cells have putative roles in tubulointerstitial inflammation in lupus nephritis (LN), but their interaction in such inflammatory process remains elusive.

Methods: CD4+ and CD8+ T lymphocytes were isolated from LN patients with (Group I) or without (Group II) moderate-to-severe tubulointerstitial inflammation, and co-incubated with PTEC in a transwell system. The level of cytokines in the culture media after 72 hours were measured and compared between Group I and II patients.

Results: Our preliminary results included 12 proliferative LN patients with (Group I; n=7) or without (Group II; n=5) moderate-to-severe tubulointerstitial inflammation. Group I patients showed significantly higher levels of RANTES (P=0.002, 0.015, and 0.014) but lower IL-10 (P=0.018, 0.015, and 0.022) when PTEC were co-cultured with their CD4+ T cells, CD8+ T cells or with both CD4+ and CD8+ T cells. Group I also demonstrated higher levels of macrophage chemoattractant protein-1 (MCP-1) [P=0.016 and 0.042] when PTEC were co-cultured with their CD8+ T cells or CD4+ and CD8+ T cells. No significant difference in IL-6 or IL-8 levels was observed, although Group I revealed numerically much higher IL-6 levels.

Conclusion: These pilot results showed a significant increase in RANTES and MCP-1 and a decrease in IL-10 levels upon interaction between PTEC and T cells obtained from LN patients with moderate-to-severe tubulointerstitial inflammation, and suggest that these cytokines might have putative in mediating tubulointerstitial inflammation in LN.

Neutrophil-derived lipocalin-2 mediates non-alcoholic steatohepatitis through modulating the cross-talk between liver and gut microbiota

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Background: Hepatic infiltration of neutrophils has been recently characterised as one of the key histological features of non-alcoholic steatohepatitis (NASH) in clinical studies, but the function of neutrophils in NASH development remains largely unknown. This study aimed to investigate the role of neutrophil-derived granule protein lipocalin-2 in mediating liver inflammation in NASH and underlying mechanisms.

Methods: Lipocalin-2-null mice were crossed with ApoE knockout mice to generate ApoE- lipocalin-2 double knockout mice and ApoE KO/ lipocalin-2 wild-type mice, which were fed with high-fat-high-cholesterol (HFHC) diet to induce NASH.

Results: Hepatic infiltration of neutrophils and lipocalin-2 expression were markedly increased in murine model of NASH. Mice lacking lipocalin-2 exhibited a significant attenuation in HFHC diet-evoked hepatocyte injury and inflammatory responses in the liver. Supplementation of lipocalin-2 with recombinant protein further boosted liver injury and metabolic inflammation in a neutrophil-dependent manner. Bone marrow transplantation-mediated alteration of lipocalin-2 in neutrophils significantly influences metabolic inflammation in mouse liver. Primary mouse neutrophils lacking lipocalin-2 showed a defective migration capacity. Mechanistically, lipocalin-2 induced phosphorylation of p38 MAP Kinase and ERK, which are mediated by activation of the chemokine receptor CXCR2 in neutrophils. Furthermore, reduced endotoxemia in lipocalin-2-null mice was significantly associated with the notably restored alteration in gut microbiome and improvement in intestine barrier functions.

Conclusions: Neutrophil-derived lipocalin-2 modulates neutrophil recruitment via CXCR2-MAPK/ERK signalling pathways and gut microbiota, thereby potentiating liver inflammation to induce hepatocyte injury in NASH.

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Characterisation of different immune cell populations in systemic lupus erythematosus pathogenesis

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Introduction: Systemic lupus erythematosus (SLE) is an autoimmune disease causing multi-organ damage and the pathogenesis involves dysregulation in multiple immune cell populations. Regulatory T cells (Tregs) are immunosuppressive and myeloid dendritic cells (mDCs) are professional for antigen presentation. It was reported that the percentages of peripheral mDCs and Tregs were decreased while monocyte-derived mDCs displayed more activated phenotypes in SLE patients. Therefore this study aimed to understand how mDCs and Tregs contribute to SLE pathogenesis.

Methods: The New Zealand Black/White F1 (BWF1) murine SLE model was used. Expression of different activation markers on splenic mDCs, the frequency of interleukin-10 (IL-10) producing splenic CD4⁺ T cells upon PMA and ionomycin stimulation and the total number of splenic Tregs were determined by flow cytometry.

Results: Splenic mDCs from symptomatic lupus mice displayed less activated phenotypes. The number of splenic CD4⁺Foxp3⁺ Tregs was also higher and further characterisation of splenic Tregs subset showed that the number of CD4⁺Foxp3⁺CD25^{low} Tregs in symptomatic lupus mice was higher. In addition, the frequency of IL-10 producing CD4⁺ T cells was higher in symptomatic lupus mice.

Conclusion: Tregs and/or other T cell sub-population(s) had displayed abnormalities in symptomatic lupus mice which suggested that they may play a role in SLE pathogenesis. More work should be done to investigate the interactions of different immune cell populations for their contribution to SLE pathogenesis.

Longitudinal study of hepatitis B virus pre-S mutations prior to development of hepatocellular carcinoma

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Introduction: Deletions/mutations in the hepatitis B virus (HBV) pre-S region have been associated with hepatocellular carcinoma (HCC). However, the evolutionary changes of pre-S mutations prior to HCC diagnosis remain unexplored.

Methods: Our study enrolled 86 CHB patients with HBV-positive serum samples available before diagnosis of HCC (HCC group). HBV pre-S sequences at 1 to 10 years preceding diagnosis of HCC were determined in HCC group. In addition, HBV pre-S sequences were determined in 146 CHB patients who were followed up at least 3 years without HCC (HCC-free group). Pre-S sequences of 47 CHB patients with earlier stored sera were longitudinally examined in HCC-free group.

Results: Compared to the HCC-free group, higher frequencies of pre-S deletions and pre-S point-mutations (at 11 codons) were observed in the HCC group (all $P < 0.05$). Multiple logistic regression analysis showed that pre-S deletions, point-mutations at codon 27, 166 and 167 were independent factors associated with HCC. Longitudinal observation showed that pre-S deletions and most of the 11 HCC-associated pre-S point-mutations existed at least 10 years before HCC development, and were more highly prevalent preceding HCC development in patients from HCC groups than HCC-free group. The number of HCC-associated pre-S point-mutations increased over time prior to HCC development, and correlated positively with the time to HCC diagnosis ($r = 0.194$, $P = 0.006$).

Conclusion: High prevalence of pre-S mutations and cumulative viral evolutionary changes preceding HCC development provide insights into the development of HCC.

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Introduction: Full-length intrahepatic hepatitis B virus (HBV) genome mutations and quasispecies characteristics in hepatocellular carcinoma (HCC) were investigated.

Methods: HBV DNA was extracted from the tumour and non-tumour tissues of 16 HCC patients. Overlapping DNA fragments covering the entire HBV genome were amplified and sequenced. To study HBV sequence at the quasispecies level, the preS region was amplified and clonal sequenced. HBV mutation profiles, quasispecies complexity and diversity, and phylogenetic characteristics were assessed.

Results: Overall, 14 patients had full-length HBV amplification. Hot-spot mutations at HBx aa130-131 and preS deletions were detected in 13 (93%) and 6 (43%) patients, respectively. Deletions in the X/preC/C regions were more frequently detected in the tumour than the non-tumour tissues (P=0.031). Compared to the non-tumour tissues, the tumour tissues had a lower quasispecies complexity (P=0.014 and 0.043, at the nucleotide and amino acid levels, respectively) and diversity (P=0.048 and 0.022, at the nucleotide and amino acid levels, respectively). Phylogenetic analysis showed that HBV sequences derived from tumour and non-tumour tissues were separately clustered, suggesting the occurrence of compartmentalisation, which was confirmed by the correlation coefficient testing on both the number and length of branches of viral populations (all P<0.02).

Conclusion: HBV mutation patterns in HCC tumour and non-tumour tissues were different. HBV quasispecies were compartmentalised, and tumour tissues had a lower genome complexity and diversity, suggesting that a selection of HBV mutations was involved in hepatocarcinogenesis.

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Introduction: White matter hyperintensities (WMH), best discerned on T2-weighted or fluid-attenuated inversion recovery magnetic resonance imaging (MRI), have been increasingly recognised as one of the underlying causes of insidious cognitive decline. However, there is little information in the literature concerning the cognitive profile of patients with such lesions. We aimed to identify and assess the spectrum of cognitive impairments associated with advanced WMH in a cohort of 340 otherwise healthy hypertensive elderly Chinese.

Methods: Demographical information, standard neuropsychological tests and multi-sequence MRI scans were obtained from all participants. The neuropsychological tests evaluated the following domains: attention, visuospatial ability, memory, language and related functions, executive function, information processing speed, and motor speed. Z score of every single test was generated from dividing the difference between individual test score and mean test score by the standard deviation. An overall Z score for multiple test domains was calculated from individual Z scores of all the component tests. WMH were evaluated using Fazekas white matter scale to generate the scores for both periventricular hyperintensities (PVH) and deep white matter hyperintensities (DWMH). Spearman correlation was used to evaluate any association between different degrees of WMH and cognitive performance in various domains. Finally, regression models were used to assess whether these associations were independent of age and education levels.

Results: Severe DWMH were negatively correlated with cognitive function on information processing speed, executive function, and motor speed. Furthermore, high PVH scores were correlated with worse performance in all test domains except language and related functions (P<0.01). After adjustment for age and education levels, severe PVH were associated with reduced information-processing speed and executive function (P<0.05).

Conclusion: Significant cognitive decline is associated with severe WMH especially PVH. Such associations with information-processing speed and executive function are independent of age and education levels.

Retrospective analysis of bevacizumab in treating KRAS-mutated type advanced colorectal cancer patients

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Introduction: Previous bevacizumab studies mainly reported the drug in untreated mutated (MT) KRAS metastatic colorectal cancer (mCRC) patients. Moreover, limited data with regard to Asian patients have been provided. Therefore, we would like to study bevacizumab efficacy based on Asian advanced CRC patients and check the consistency with historical data.

Methods: Between 2008 and 2012, MT KRAS mCRC patients who received bevacizumab-containing regimens at Queen Mary Hospital were reviewed. Overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) were assessed to compare with historical data.

Results: Overall, 36 patients were included in the analysis. ORR reached 51.5% for first-line bevacizumab, and dropped to 48.6% when included second-line treatments. Median PFS was 8.9 months with respect to first-line, and 10.1 months if including second-line treatments. Median OS reached 18.1 months for first-line patients, and 18.4 months for all assessed patients.

Conclusions: The clinical efficacy and toxicity of bevacizumab in treating Asian advanced CRC patients with mutated KRAS status were consistent with previous studies.

Retrospective analysis of cetuximab in treating Hong Kong advanced colorectal cancer patients

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Introduction: Limited data of cetuximab in treating advanced Asian colorectal cancer (CRC) patients have been reported. Moreover, no retrospective or prospective analysis has compared the efficiency of cetuximab in different lines of treatment. Therefore, we would like to study about the clinical use of cetuximab in treating Asian patients and compare with historical data.

Methods: Between 2008 and 2012, metastatic CRC patients who received cetuximab-containing regimens at Queen Mary Hospital were reviewed. Overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) were assessed to compare with historical data.

Results: Overall, 52 patients were included in the analysis. ORR reached 71.8% for first-line cetuximab, and dropped to 67.3% when included second-line and third-line treatments. Median PFS was 8.9 months with respect to all lines of treatment. Median OS reached 27.5 months for all patients. No significant difference with respect to PFS and OS was observed when comparing patients in first-line and further lines.

Conclusions: The clinical efficacy and toxicity of cetuximab in treating Asian advanced CRC patients were consistent with previous studies.

Comparison of cetuximab and bevacizumab as first-line treatment in KRAS wild-type advanced colorectal cancer patients: a retrospective analysis

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Introduction: Cetuximab and bevacizumab-based therapies have been demonstrated to improve survival outcomes as first-line treatment in wild-type (WT) KRAS metastatic colorectal cancer (mCRC) patients. However, few studies have evaluated any difference in treatment outcomes between cetuximab and bevacizumab-based therapies.

Methods: Between 2008 and 2012, KRAS WT mCRC patients who received cetuximab-containing or bevacizumab-containing regimens as first-line treatment at Queen Mary Hospital were reviewed. Overall response rate (ORR), progression-free survival (PFS), and overall survival (OS) were assessed as the study endpoints in the analysis.

Results: Overall, 50 patients were included in the analysis. ORR was seemingly higher in cetuximab arm (77.8%) as compared to bevacizumab arm (53.3%), though not statistically significant ($P=0.08$). Similar median PFS (9.6 vs 9.7 months) and median OS (27.5 vs 27.4 months) were reported between arms. Second-line therapy was given in 82.9% and 80% of patients, respectively.

Conclusions: Comparable ORRs, PFS, and OS were observed, which might suggest similar efficacy and survival outcomes of upfront cetuximab-based and bevacizumab-based chemotherapies in treating KRAS WT mCRC patients.

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

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Background: Type 2 diabetes mellitus (T2DM) complicated by retinopathy is associated with impaired left ventricular (LV) structure and resting myocardial dysfunction. The myocardial response to stress has not been compared in patients with and without diabetic retinopathy.

Methods: A total of 134 patients with T2DM without any history of cardiovascular disease were recruited. All patients underwent detailed retinal photography to screen for diabetic retinopathy, resting and exercise echocardiography. Resting echocardiography was analysed by (1) conventional echocardiographic parameters and (2) speckle tracking derived global longitudinal strain (GLS). Exercise echocardiography parameters included diastolic function reserve index (DFRI) and stress GLS.

Results: A total of 43 (23%) patients had retinopathy. For resting echocardiography, both LV dimension and LV ejection fraction were similar between patients with and without diabetic retinopathy. However, patients with retinopathy had a significantly impaired GLS, higher prevalence of diastolic dysfunction, a higher E/E' ratio (LV filling pressure) compared with patients without retinopathy. Stress echocardiography also showed that patients with diabetic retinopathy also had a more impaired DFRI and stress GLS. Multivariable analysis showed that the presence of diabetic retinopathy was independently associated with a higher resting E/E', diastolic dysfunction grade, an impaired resting GLS, a low DFRI and impaired stress GLS.

Conclusion: Patient with T2DM and retinopathy had impaired (i) resting myocardial function (diastolic function and GLS) and (ii) stress myocardial function (DFRI and Δ GLS) compared to those without retinopathy. Such data thus suggested that microvascular dysfunction contributed to both resting and stress myocardial dysfunction in patients with T2DM.

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Neutrophil serine proteases as early mediators in autoimmune diabetes

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Introduction: Type 1 diabetes (T1D) is an autoimmune disease resulting from self-destruction of insulin-producing beta-cells. Increased neutrophil serine proteases (NSPs) have recently been implicated to be closely associated with beta-cell autoimmunity in patients with T1D. In this study, we used the non-obese diabetic (NOD) mice and a T1D animal model to investigate how NSPs participate in the T1D pathogenesis.

Methods: Circulating protein levels and enzymatic activities of neutrophil elastase (NE) and proteinase 3 (PR3) were measured weekly in NOD mice from 2 to 30 weeks of age. Elafin, the endogenous inhibitor of NE and PR3, was expressed specifically in the islet of NOD mice via adeno-associated virus system and the chemical inhibitor sivelestat was injected into NOD mice intraperitoneally. T1D incidence was compared in NOD mice with or without inhibitor treatment. Flow cytometry and immunohistochemistry (IHC) were applied to assess insulinitis severity, quantify the early pancreas-infiltrating immune cells and intra-islet cytokine expression. Pro-inflammatory cytokine expression levels were determined by quantitative polymerase chain reaction and western blotting in min6 beta-cells and RAW264.7 macrophages treated with pbs or NE protein. The chemotaxis assay was used to measure the macrophage migration.

Results: A significant increase in circulating NE and PR3 protein levels and enzymatic activities were observed from 3-week-old to 6-week-old NOD mice. Therapeutic intervention with the inhibitor elafin or sivelestat at early stage largely reduced the T1D incidence in NOD mice. IHC and flow cytometry analysis revealed that inhibition of NE and PR3 attenuated macrophage infiltration, reduced iNOS production, and decreased insulinitis in the pancreas of NOD mice. Treatment of NE protein directly elevated tumour necrosis factor- α and interleukin-1 β expression in both Min6 and RAW cells via phosphorylation of I κ B α which led to NF- κ B activation. Furthermore, conditioned medium from NE-treated min6 cells significantly enhanced the migration of RAW264.7 macrophages.

Conclusion: Collectively, these results demonstrate that NSPs (NE and PR3) may serve as a potent link in mediating the crosstalk between innate immune cells and NE and PR3-induced early pro-inflammatory responses in beta-cells trigger macrophages recruitment which in turn contributes to beta-cell autoimmunity in NOD mice.

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