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Oral Presentations

Session I

16 August (Friday), 9.00-10.30am

The interplay between O-GlcNAc and phosphorylation on tyrosine hydroxylase activity and cathecolamine synthesis in PC12 cells

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The Hexosamine biosynthetic pathway uses about 2 % to 5 % of all glucose that enters the cell, having the glutamine fructose-6-phosphate aminotransferase (GFAT) as the rate-limiting enzyme that converts fructose 6-phosphate into glucosamine-6-phosphate. This pathway has UDP-GlcNAc as final product, which is used as substrate for intracellular O-GlcNAc; a post-translational modification (PTM) resulting from the covalent addition of a N-acetylglucosamine (GlcNAc) to the hydroxyl groups on serine residues and/or threonine proteins. This reaction is catalyzed by O-GlcNAc transferase (OGT), and the removal reaction of this monosaccharide is made by O-GlcNAcase (OGA). The balance of the activity of such enzymes will regulate the levels of O-GlcNAcylated proteins, with an O-GlcNAcylation, similarly to phosphorylation, highly inducible, dynamic and active in many cellular processes. The tyrosine hydroxylase (TH) is an enzyme responsible for catalyzing the rate-limiting step in catecholamine synthesis hydroxylating L-tyrosine at position meta to obtain dihydroxyphenylalanine (L-DOPA). There is only one evidence in the literature that TH is O-GlcNAcylated, and that decreased levels of this post-translational modification stimulates the secretion of dopamine in PC12 cells (rat pheochromocytoma); however, the mechanism of how this actually occurs remains unknown. From this, using the pharmacological inhibitor of OGA Thiamet G (TMG); and nerve growth factor (NGF), a compound that induces neuritogenesis and enhances TH activity on PC12 cells, our group showed by Western Blotting analyses that O-GlcNAcylation acts on the control of the phosphorylation levels of serine 40 in TH, where stimulation by 28 % on the increase in phosphorylation at serine 40 drops in 26 % the levels of O-GlcNAc compared to control; while the increasing of intracellular O-GlcNAc in 19 % reduces the phosphorylation at serine 40 by 16 %. In addition, HPLC analysis shows that increasing the intracellular O-GlcNAcylation reduce the levels of dopamine by 80 %. Finally, an *in vitro* reaction between OGT, TH recombinant enzyme and UDP-GlcNAc shows that, indeed, TH suffers this PTM. These data suggest a mechanism that integrates carbohydrate metabolism (by HBP) with the catecholamine biosynthetic pathway; where competition between O-GlcNAc and phosphate at serine 40 tyrosine hydroxylase site modulates its activity, controlling the synthesis of dopamine levels in PC12 cells.

Key-words: O-GlcNAc, tyrosine hydroxylase, dopamine

Metabolic status of adipose tissue after fructose overconsumption – differences between young and adult female rats

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Modern diet, rich in refined sugars and sweeteners, led to dramatic increase in fructose intake especially in young population. Excessive fructose intake has been associated with growing rate of obesity, insulin resistance and development of metabolic syndrome, women being more prone than men. Chronic low-grade inflammation accompanies obesity and has been implicated in the pathogenesis of obesity-related disorders including metabolic syndrome and insulin resistance. To elucidate whether fructose overconsumption causes inflammation in the visceral adipose tissue (VAT) of young and adult female rats thus contributing to the development of obesity and insulin resistance. We investigated the effects of 9-week fructose-enriched diet applied immediately after weaning (young) or at the 2.5 months of age (adult) on nuclear factor κB (NF-κB) intracellular distribution, and on the expression of pro-inflammatory cytokines (IL-1β, IL-6 and TNFα) in female Wistar rats. Additionally, insulin signalling was analysed at the level of insulin receptor substrate-1 (IRS1), Akt kinase, and their activating and inhibitory phosphorylations. Fructose-enriched diet increased absolute and relative VAT mass in young female rats. There were no changes in VAT mass of adults after fructose diet, even though histological analysis revealed the presence of islets of smaller adipocytes, which indicated adipogenesis. Both young and adult female fructose-fed rats had increased nuclear accumulation of NF-κB and elevated expression of pro-inflammatory cytokines in the VAT. In adults, fructose overconsumption reduced protein content and stimulatory phosphorylation of Akt kinase, while increasing inhibitory phosphorylation of IRS-1. These changes were not observed in young female rats. The results suggest that fructose-enriched diet causes inflammation in VAT of both young and adult female rats. Our work supports the stand that VAT inflammation could represent one of the earliest metabolic perturbations upon fructose overconsumption, since it can occur even before the onset of obesity or insulin resistance. Additionally, only adults developed VAT insulin resistance, indicating age-dependent differences in insulin signalling system and its response to fructose overconsumption in female rats.

Key words: inflammation, insulin resistance, fructose, adipose tissue, female rats

Computer-aided synthesis of novel 'phytochemical-sulfonamide' hybrids as prospective alternate anti-MRSA drugs

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The Gram-positive bacterium, methicillin-resistant *Staphylococcus aureus* (MRSA) causes common place infection at hospital and community sectors, with simultaneous aggrandizement of resistance to several antibiotics, resulting in additional morbidity and unexpected mortality. As a solution to this problem, herein designing of six novel 'phytochemical-sulfa drug' hybrid agents using azo-coupling synthesis was done. Bioinformatics tools such as PASS prediction, Lipinski rules of five, computational LD50 value, toxicity class were employed to assess the drug-likeliness properties, before synthesis. The three-dimensional enzyme structure of the target, dihydropteroate synthases (DHPS) of MRSA was generated and validated by Ramachandran plot. Additionally, molecular docking, dynamics simulations study was used for intermolecular interactions and stability of DHPS with the designed conjugates. After that, most effective conjugates were synthesized and characterized by advanced spectral techniques such as UV, FTIR, LCMS, HPLC, NMR, and SEM. The zone of inhibition, MIC and MBC values of conjugates were determined against isolated MRSA strains from clinical samples with *in vitro* study. Conjugates, **4b** (thymol-sulfadiazine) and **4d** (thymol-sulfamethoxazole) had the highest zone size of inhibition on agar plates with 20 and 40 μg/mL as the lowest MIC and MBC values against MRSA, respectively; while the reference antibiotic ampicillin had the most inferior MIC and MBC values at 80 to 180 μg/mL. *In vitro* host-toxicity testing of **4b** and **4d** with cultured human lymphocytes grown from umbilical cord blood confirmed that, broadly non-toxic to human cells and could be promoted as newer antibacterial agent against gruesome MRSA and other MDR pathogenic bacteria.

Keywords: phytochemical-sulfadrug conjugates, MRSA-DHPS, molecular docking simulation, host-toxicity with cultured human lymphocytes

Structural analysis of ligands entrance mechanism of peroxisome proliferator-activated receptors δ (PPARδ)

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Peroxisome proliferator-activated receptors (PPARs) are family of nuclear receptors belongs to the zinc-finger type transcription factors and plays a critical role in controlling fatty acid metabolism and catabolism for energy homeostasis by inducing the expression of genes involved in biosynthesis, oxidation, storage, and transport of lipid. The PPAR family contains three subtypes: PPARα, PPARγ and PPARδ (PPARβ). PPARα and PPARγ are the molecular targets of a number of marketed drugs for treating hyperlipidemia, insulin resistance, and other diseases. While no synthetic PPARδ agonists are yet approved for human use, PPARδ was reported to be involved in fat burning, which demonstrates that PPARδ agonists should be potential therapeutic agents in treating obesity and diabetes. Much efforts to develop improved PPARδ agonists have been based on early synthetic PPARδ agonists characterized phenoxyacetic acid derivatives such as GW501516 and GW0742. Recently, PPARδ has been suggested as a direct target of DrugX (*unpublished data*). Curiously, DrugX is a compound which displays a chemical structure distinct from those of phenoxyacetic acid derivatives. Here I showed the crystal structures of the ligand binding domain (LBD) of human PPARδ complexed with DrugX at high resolution (2.00 Å). The structure of PPARδ LBD drugX complex consists of four shorts stranded β-sheet and a bundle of 12 α helices. I found that DrugX is located inside of the ligand-binding cavity and interacts to PPARδ LBD via multiple hydrogen bonds formed by the guanidino group of the DrugX with polar residues, together with contacts with nonpolar residues. The structural comparison with structures of PPARδ LBD bound to phenoxyacetic acid derivatives clarifies that the hydrogen bonding interactions between the DrugX guanidino group and PPARδ LBD unexpectedly exhibit a similar fashion to the carboxylates group of phenoxyacetic acid derivatives, although the chemical structures are dissimilar to each other. This finding leads to a deeper understanding of the specificity of the PPARδ agonists and provides valuable structural information for potential next-generation drug design in treating metabolic syndromes, including diabetes mellitus.

Polyphenol-rich extract of *Bulbine natalensis* and *Bulbine fructescens* stimulate insulin secretion from pancreatic MIN6 β-cells, mouse islets and enhanced glucose utilisation

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Bulbine natalensis (BN) and *Bulbine fructescens* (BF) are recommended in South African folk medicine as ingredients of polyherbal therapy used to treat diabetes mellitus, but their therapeutic targets have not been elucidated. For this study, the phenolic acid profiles, mineral composition and anti-oxidant capacity of the plant extracts, and their inhibitory potential against alpha-amylase, alpha-glucosidase and pancreatic lipase, were evaluated. The *in vitro* effects of methanol extracts of BN and BF on insulin secretion from MIN6 β-cells and isolated mouse islets, and on glucose utilisation in human liver Huh-7 cells were also investigated. The results revealed that methanol crude extracts of BN and BF contained total phenolic contents of 667.0 and 767.8 mg gallic acid equivalents/g, respectively. The plant extracts contained high concentration of protocatechuic and gallic acids, while vanillic acid was not detected. Mineral composition analysis of both plant extracts using ICP-OES indicated substantial concentrations of Zn, Mn, Ca and K. BN and BF extracts robustly inhibited pancreatic lipase, alpha-glucosidase and alpha-amylase activities as well as DPPH radical scavenging activity. In addition, both extracts significantly stimulated insulin secretion from MIN6 β-cells and isolated mouse islets at 0.1 mg/mL and 1 mg/mL, without compromising membrane integrity and they increased glucose utilisation at 12.5 μg/mL. In summary, we have shown that methanol extracts of these botanicals directly stimulate insulin secretion from mouse islets, MIN6 β-cells and enhance glucose utilisation, possibly due to the presence of minerals and phenolic acids with proven anti-diabetic potential.

Structural and functional insight into the bioplastic-producing enzyme – PHA synthase

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PHA synthase (PhaC) is the key enzyme in the biosynthesis of a type of biodegradable polymers, polyhydroxyalkanoate (PHA), by polymerize the acyl group of the acyl- coenzyme A into a high molecular weight polymer with the concomitant release of CoA. Most bacteria can synthesize PHA when there are plenty of carbon but limited amount of other nutrients in surrounding environment. As the pollution from single-use plastics worsened in recent days, PHA can serve as a promising alternative material to replace the conventional petrochemical plastics. Over the years, PHA has shown its advantageous characters in elasticity and thermostability, which is similar to conventional petrochemical plastics, with the advantage of being biodegradable and able to synthesize from renewable resources. The crystal structure of the C-terminal catalytic domain of PhaC from *Chromobacterium* sp. USM2 (PhaCc_s-CAT) was determined (1.48 Å). The free form structure displayed α/β hydrolase core subdomain and a CAP subdomain. In the CAP subdomain, a sl1ort 'LID region' covers the catalytic triad comprised of Cys29l, Asp447, and His477, blocks the substrates from entering to the active site. The water molecules present in the active site indicates that the CAP subdomain is flexible and can serve as the substrate entry point. In addition, the structure showed PhaCc_s-CAT is a dimer, which is a general depicted biological active conformation. The crystal structures of the catalytic domain of PhaC from *Cupriavidus necator* (PhaCc_n-CAT) determined by two other research groups also displayed dimeric arrangement (1.8 Å). While the exact proposed mechanism is still debatable due to the lack of the structure of the N-terminal domain, the crystal structures of PhaCc_s-CAT and PhaCc_n-CAT favour the processive polymerization model instead of the non-processive ping-pong polymerization model. Furthermore, the functional roles of the residues in the beneficial mutations can be explained based on the crystal structures. In summary, the closed form PhaCc_s-CAT, together with the partially open-form PhaCc_n-CAT, provide us insights on how the enzymes polymerize the biodegradable polymers. However, more efforts have to put into understand the substrate specificity of this enzyme, which is important for synthesizing the practical materials with the right type of PHA co-polymers, such as poly[3-hydroxybutyrate-co-3-hydroxyheaxanoate].

Keywords: Polyhydroxyalkanoate, PHA synthase, biodegradable, bioplastic, *Chromobacterium* sp. USM2

Isolation and characterization of compounds from *Pleurotus ostreatus* and evaluation of anti-inflammatory activity

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Pleurotus ostreatus (P.o) is a culinary-medicinal mushroom grown worldwide. We have reported the anti-inflammatory potential of the aqueous fraction of acetone extract (AqFrA) of P.o and its sub fractions obtained by size exclusion chromatography (Fr_a, Fr_b, Fr_c and Fr_d), normal phase column chromatography (Fr_1, Fr_2, Fr_3, Fr_4 and Fr_5) and silica gel column chromatography (Fr_{1-1}, Fr_{1-2}, Fr_{1-3} and Fr_{1-4}). This study evaluates the isolation and characterization of compounds from different sub fractions and determination of anti-inflammatory activity of compounds. Anti-inflammatory activity of each compound was measured using carrageenan induced rat paw oedema model. The effect on reactive oxidative burst of human whole blood was measured by luminol-enhanced chemiluminescence activity. Fr_{1-1} and Fr_2 were further subjected to Sephadex LH 20 column and resulted sub fractions were subjected to recycling size exclusion HPLC, which yielded two pure compounds **G** and **5** respectively. The Fr_d was subjected to recycling reverse phase HPLC, yielded one pure compound (**A**). Fr_c and remaining Fr_d were combined and chromatographed over silica gel. Sub fractions subjected to recycling size exclusion HPLC yielded pure compounds (**D1**) and (**C2**). The 1H NMR spectrum, 13C NMR spectrum, 2D COSY, NOESY, HSQC, HMBC spectra and the EI+ /ESI mass spectra were recorded. Structure elucidation revealed that compounds **A**, **D1** and **C2** were N10 isopentenyl adenosine, uracil and niacinamide respectively. Compound **5** and **G** speculated to be a derivative of uridine and a disaccharide. N10 isopentenyl adenosine and the derivative of uridine showed more than 50 % inhibition on whole blood oxidative burst (74.6 % and 51.2 % respectively). Treatment with compounds N10 isopentenyl adenosine, derivative of uridine and indomethacin showed significant ($p<0.05$) inhibition of rat paw oedema when compared with the control group. The anti-inflammatory activity of N10 isopentenyl adenosine was comparable with that of indomethacin at 4th and 5th hours. N10 isopentenyl adenosine, the derivative of uridine and indomethacin showed maximum inhibition of oedema of 83.8 %, 92.3 % and 86.5 % respectively ($p<0.05$). In conclusion, the derivative of uridine and novel isolated compound N10 isopentenyl adenosine possess significant ($p<0.05$) anti-inflammatory activity in rats.

Key words: *Pleurotus ostreatus*, anti-inflammatory, oxidative burst, isopentenyl adenosine

Oral Presentations

Session II

16 August (Friday), 11.00am-12.30pm

RASSF6 tumor suppressor protein regulates apoptosis and cell cycle progration via retinoblastoma protein

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RASSF6 is a member of the tumor suppressor Ras-association domain family (RASSF) proteins. RASSF6 expression is epigenetically suppressed in human cancers and its low expression is associated with poor prognosis. RASSF6 plays a tumor suppressor role by regulating cell cycle arrest and apoptosis. Mechanistically, RASSF6 blocks MDM2-mediated p53 degradation and enhances p53 expression. However, RASSF6 also induces cell cycle arrest and apoptosis in the p53-negative background, which implies that the tumor suppressor function of RASSF6 does not solely depend on p53. In this study, we have revealed that RASSF6 enhances the interaction between pRb and protein phosphatase and induces CDKN2A. In this way, RASSF6 increases unphosphorylated-pRb and augments the interaction between pRb and E2F1. Moreover, RASSF6 increases *TP73*-target genes via pRb and E2F1. Finally, we found that RASSF6 depletion induces polypoid cells in the p53-negative background. In conclusion, RASSF6 plays as a tumor suppressor in cancers with the loss-of-function of p53 and pRb is implicated in this function of RASSF6.

Keywords: RASSF6, RB1, apoptosis, cell cycle arrest, tumor suppressor

The genome wide study of estrogen receptor alpha (ERα)-regulated enhancers characterization by glucocorticoid receptors (GR) in breast cancer

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Genome-wide transcriptional responses are orchestrated by a large network of genomic enhancers. Despite their discovery more than 35 years ago, many of the fundamental principles by which enhancers are activated and regulate their transcriptional target coding genes in metazoans remain poorly understood. The wide diversity of cell status, each exhibiting precisely regulated gene expression, requires a highly-coordinated network of regulatory enhancers, in which activation or repression of single element is fine-tuned by a variety of external signals to ensure homeostasis. Disturbance of the enhancer regulatory network, including epigenetic reprogramming and/or genomic mutations in noncoding regulatory enhancer regions, has been linked to multiple diseases, including cancer. Here, I am specifically focusing on enhancer regulatory mechanisms that contribute to the progression of ER-positive breast cancer from estrogen-dependent to acquired hormone-refractory stage, including 17β-estradiol (E2)-regulated ERα-enhancer network in sensitive state and hormone-independent activation of alternative enhancer networks in resistant state. I combine contemporary machine learning approaches and advanced global genomic technologies to systematically decipher enhancer regulatory mechanisms that contribute to the progression of ER positive breast cancer from estrogen-dependent to acquired hormone-refractory stage, with deeper insights into previously-ignored functional cross-regulatory network among distal enhancers. It aims to use computational approaches, based on integration of epigenetic profiling and RNA expression, to unbiasedly uncover key transcriptional factors and/or co-regulators that are critical for acquired drug resistance to endocrine therapy. Further, to delineate the heterogeneous resistant mechanisms, this study will focus on tracing the dynamic molecular events (enhancer landscape and transcriptome) during the transition course, from sensitive to resistant stage, using machine-learning coupled scATAC-seq and scRNA-seq at series of time points following drug treatment that could be therapeutic indication for treatment in acquired resistance of endocrine therapy in ER+ breast cancer.

Heat-activated retrotransposons in the algal symbiont of corals, *Symbiodinium microadriaticum*

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The phenomenon known as Coral Reef bleaching is caused by the loss of symbiotic algae known as Zooxanthallae or *Symbiodinium* from the tissue of coral polyps. This sudden loss is triggered by environmental stressors, the most common of which is heat stress. The molecular mechanism by which this process occurs is still an active area of research, and we therefore carried out a transcriptomics study on free-living *Symbiodinium* cultures to establish a base-line understanding of gene expression changes under heat stress. We report that the two most highly upregulated of the handful of genes that showed differential expression during heat stress in our dataset were annotated as retrotransposons. We subsequently isolated and identified four *Symbiodinium microadriaticum* Ty1-Copia-like (SmTCLs) retrotransposons whose expression was found to be upregulated under heat stress. We present evidence to suggest that these are full length Long Terminal Repeat (LTR) retrotransposons, and are part of a family of retrotransposons that have undergone an evolutionarily recent expansion in copy number in the *S. microadriaticum* genome.

Genome-wide analysis of the Dof gene family in durian reveals fruit ripening-associated and cultivar-dependent Dof transcription factors

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Fruit ripening is a coordinated developmental process regulated by transcriptional networks. DNA binding with one finger (Dof) proteins constitute a ubiquitous plant-specific transcription factor (TF) family associated with diverse biological processes, including ripening. However, our knowledge of Dof TFs in fruits of economic interest is relatively limited. We conducted a genome-wide analysis of durian (*Durio zibethinus* Murr.) and identified 24 durian *Dofs* (*DzDofs*), 15 of which were expressed in the fruit pulp. Gene expression analysis revealed differential expression of *DzDofs* during ripening in two commercial durian cultivars from Thailand, Monthong and Chanee. Seven ripening-associated *DzDofs* were identified in both cultivars. Comparing the expression levels of fruit pulp-expressed *DzDofs* between these cultivars revealed ten potential cultivar-dependent Dofs, among which *DzDof 2.2* showed a significantly greater fold increase at every ripening stage in Chanee than in Monthong. The prediction of DzDof2.2's function based on its orthologue in *Arabidopsis* revealed its possible role in regulating auxin biosynthesis. We observed a positive correlation between levels of *DzDof2.2* expression and indole-3-acetic acid accumulation in both cultivars. Higher auxin levels in Chanee could activate auxin-mediated transcription, contributing to the faster ripening of this cultivar compared to Monthong through earlier initiation of the ethylene response (auxin-ethylene crosstalk).

Genetics of Alzheimer disease in Asia

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Alzheimer disease (AD) is a very common progressive neurodegenerative disorder, characterized by gradual loss of memory, decline in other cognitive functions and decrease in functional capacity. Molecular analyses of families with early onset of AD (EOAD) made possible to identify dominantly acting mutations in genes, such as amyloid precursor protein precursor protein (APP) and presenilin 1 and 2 (PSEN 1 & PSEN 2). However, the etiology of the late onset of AD (LOAD) was less straightforward than EOAD. In most Asian countries, the population and the number of AD patients are growing rapidly, highlighting the needs to colligate mutations across populations. DNA extraction, linkage study, whole exome sequencing, next-generation sequencing and Sanger sequencing, bioinformatics, neurological evaluation, diagnostic imaging, *APOE* gene polymorphism analysis, and pathological assessment were performed in all patients. We reported the results of genetic analysing from large-scale EOAD series ascertained across four Asian countries, Thailand, Malaysia, Philippines and Korea, from 2009 to 2019. Over 400 patients, familial and sporadic cases were screened, 40 cases carried a mutation in *APP*, *PSEN1* and *PSEN2*, which had all three CSF biomarkers—total tau protein (t-Tau), phospho-tau protein (p-Tau), and amyloid β (Aβ) 42—in abnormal ranges. Among the 40 distinct mutations found in the patients and isolated cases in 4 populations, definite pathogenicity established for only 10 %, emphasizing the needs to survey variants in larger patient cohorts. Currently, we are exploring their causative functional effects by cloning them into stable cell line. In addition, the segregation analyses in the family members, as well as targeting deep resequencing in large datasets for validating the role of the variants are in works.

Key words: Alzheimer disease, genetic, Asian, sequencing, mutation

Osteogenic differentiation of human mesenchymal stem cells in three dimensional collagen-based microenvironment

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Increased knowledge and technology in the field of stem cells accelerates the development of regenerative medicine. Mesenchymal stem cells (MSCs) are multipotent cells capable to differentiate into several types, including osteocytes, chondrocytes (cartilage cells), muscle cells, fat cells and others. The MSC's multipotent ability attracts our attention for its prospective function in the field of regenerative medicine, including for bone malformation. Studies in animals have tried to succeed in mesenchymal stem cell therapy to resolve bone fracture healing. Although the ability of osteogenic differentiation of mesenchymal stem cells has been demonstrated both *in vivo* and *in vitro*, the use of mesenchymal stem cells in clinical applications still requires further studies to improve effectiveness. In the current study, we evaluated the osteogenic differentiation of human MSCs in 3D collagen capsule. MSCs were derived from human umbilical cord and cultured in platelet lysate supplemented media. The microencapsulation of stem cells was originally developed in our laboratory and become a functional tool to study the effect of 3D microenvironment for stem cell differentiation. The expression of bone related genes including ColIα1 (collagen type I α1), AKP2 (alkaline phosphatase 2), IBSP (integrin-binding sialoprotein), Runx2 (Runt-related transcription factor 2), OCN (osteocalcin) in microencapsulated cells were evaluated on day 7 post-encapsulation in 3D environment. Moreover, alizarin red staining was conducted to visualize the osteogenic cells. This study is expected to shed light on the relation of 3D microenvironment for osteogenic differentiation of MSCs.

Keywords: mesenchymal stem cells, osteogenic differentiation, 3D niche, microencapsulation

Development and characterization of Gantrez® AN119 nanoparticles loading cyclosporine A to oral administration – *In vivo* behavioural studies

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Immune system changes play key role in many disorders and there is growing demand to discover novel immunosuppressant's. Cyclosporine A (CsA) has been widely used as a potent immunosuppressive agent in spite of its low oral bioavailability and formulation problems. In the present study, we focused to develop 13 novel nano-formulations containing CsA associated to PVM/MA nanoparticles by inclusion of cyclodextrin derivative or poly(ethylene glycol) 2000 (PEG2000) and characterized it in order to select the better nano-formulation. Formulation A, B, C and D showed best physicochemical properties and were evaluated *in vivo* in order to describe their behaviour. The CyA nanoparticles were analysed by HPLC for yield, stability, encapsulation efficiency and *in vitro* release; dynamic light scattering studies for particle size and potential charge; and thermal analysis, X-Ray, FITR for solid-state characterization. The bioavailability of CyA nanoparticles were evaluated in rats, compared with the current available CyA microemulsion (Neoral®) and their *in vivo* behaviour was described. The addition of PEG2000 and derivative cyclodextrin to the formulation of PVM/MA nanoparticles increased the efficiency of CsA encapsulation. Formulations A, B, C and D show uniform sizes and zeta potentials for an efficient interaction with the mucosa of the gastrointestinal tract. Stability studies of Formulation B, C and D show that these systems are stable at 5 °C during at least 1 year. When Formulation B is administrated oral in rats, we has been found drug bioavailability in magnitude increase 21.43 % that when administered in the oral formulation Sandimmun Neoral®, on the other hand when the drug is administrated in formulations C and D cyclosporine the bioavailability is similar to the commercial formulation. In addition, when Formulations B, C and D are administrate in rats absorption of cyclosporin A is slower than commercial formulation, which results in blood drug levels sustained over time. The results suggest that CsA PVM/MA nanoparticles remained at the absorption site in the gastrointestinal tract for a period and did not pass into the systemic circulation. These novel nanoparticles can carry CsA to the surface of the intestinal mucosa where they remain in contact with the membrane of the enterocyte and release their content. Components of novel formulations can facilitate drug absorption due to the inhibitory properties of PEG2000 and derivative cyclosdextrin on the P-glycoprotein and cytochrome P450, resulting in a decreased gut-wall metabolism of CsA. Our results suggest that formulation B, C and D can be an alternative to commercial formulations of CsA for oral administration without the adverse effects caused by the vehicle, Cremophor® EL and formulation B increase drug bioavailability.

Oral Presentations

Session III

17 August (Saturday), 9.00-10.30am

Dengue virus specific NS3-RNA interactions

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The protein-RNA interactions within the flavivirus replication complex (RC) are not fully understood. Our structure of dengue virus NS3 adenosine triphosphatase (ATPase)/helicase bound to the conserved 5' genomic RNA 5'-AGUUGUUAGUCU-3 ' reveals that D290 and R538 make specific interactions with G2 and G5 bases respectively. We show that single-stranded 12-mer RNA stimulates ATPase activity of NS3, however the presence of G2 and G5 leads to significantly higher activation. D290 is adjacent to the DEXH motif found in SF2 helicases like NS3 and interacts with R387, forming a molecular switch that activates the ATPase site upon RNA binding. Our structure guided mutagenesis revealed that disruption of D290-R387 interaction increases basal ATPase activity presumably as a result of higher conformational flexibility of the ATPase active site. Mutational studies also showed R538 plays a critical role in RNA interactions affecting translocation of viral RNA through dynamic interactions with bases at positions 4 and 5 of the ssRNA. Restriction of backbone flexibility around R538 through mutation of G540 to proline abolishes virus replication, indicating conformational flexibility around residue R538 is necessary for RNA translocation. The functionally critical sequence-specific contacts in NS3 RNA binding groove in subdomain III reveals potentially novel allosteric anti-viral drug targets. Our recent studies investigating binding affinities aim to exploit this interaction to stabilise the NS3-NS5 complex for structural studies.

Keywords: dengue virus, helicase, NS3, RNA-interactions

Artemisone, a potent pan-reactive antimalarial that synergizes redox imbalance in *P. falciparum* transmissible gametocyte stages

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Malaria remains the most important parasitic disease, due to a high disease incidence and mortality associated with a remarkable loss in annual GDP. Although, chemotherapy, coupled with vector control, has reduced malaria disease mortality remarkably, emerging drug resistance towards currently used Artemisinin combination therapies, warrants the need for novel antimalarial therapies. These therapies should not only target the proliferative asexual stages, but also the transmissible gametocyte stages of *Plasmodium falciparum* parasites to block transmission of parasites between the host and mosquito. In addition, novel combination therapies should have activity against current field resistant strains as well as slow down the rate of resistance formation through targeting novel drug targets within *P. falciparum* parasites. Artemisinins are oxidant-active drugs through their bio-reduction and release of labile oxygen, oxidising reduced flavin cofactors of flavin disulphide reductases, which are essential for maintenance of redox-homeostasis in *P. falciparum* parasites. Using a novel artemisinin derivative, artemisone, we showed potent activity against asexual proliferative and sexual transmissible gametocyte stages of the parasite. Albeit with slower kill-kinetics against mature stage gametocytes compared to the asexual stages, this pan-reactivity makes these prerequisite drugs for blocking transmission of the parasite to the mosquito. Furthermore, co-treatment of this compound with a pro-oxidant redox partner drugs such as methylene blue (MB) and Naphthoquinone derivatives, showed notable synergism particularly against mature gametocyte stages. Therefore, the induction of oxidative stress by artemisone is sustained and even enhanced by the redox cycling action of these partner drugs in mature gametocytes. Thus, we show that redox-homeostasis is essential to maintain gametocyte viability prior to transmission, making it an ideal and novel drug target in *P. falciparum* parasites. Through utilizing these redox-active coupled partner drugs, that are dual-active against the proliferative and gametocyte stages, we aim to further develop a novel triple drug combination strategy through combining the redox-active drugs with a novel third partner drug with a different mechanism of action. Through this triple drug combination approach it will enable the ability to target multiple stages of the parasite's life-cycle for effective disease treatment, blocking of parasite transmission whilst limiting drug resistance formation.

Local host immune analysis during systemic *Candida albicans* infection in an intravenous challenge mouse model

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Systemic candidiasis remains the cause of high mortality and morbidity, particularly in immunecompromised populations. Under this circumstance, host-*Candida* interaction is imperative in determining the outcome of infection and to unravel the molecular pathogenesis underlying *C. albicans* infection. Thus, this study had undertaken an intravenous challenge mouse model to investigate the host response during systemic *C. albicans* infection. In this study, BALB/C mice were injected intravenously via lateral tail vein with lethal dose of clinical *C. albicans* isolate (HVS6360). Various parameters including survival study, fungal burden test and histopathological analysis of affected organs were adopted to measure the severity of the infection. Meanwhile, local host response against *C. albicans* were examined via PCR array, cytometry bead array (CBA) and ELISA analysis. The findings in this study indicate that mice infected with *Candida albicans* resulted in high mortality rate. Susceptibility of mice correlates with high fungal loads in kidneys and brains, infiltration of leukocytes (especially neutrophils) and tissue pathology with moderate to severe inflammation seen in kidney and brain tissues. On the other hand, pattern recognition receptors (PRRs), chemokines and cytokines profiles were varied among kidney, spleen and brain over 72 h post infections. Besides that, organ-specific protein expression profiles of cytokines and chemokine through cytometry bead array (CBA) and ELISA analysis revealed that local cytokine profiles in brain and kidney were similar, which mainly involved pro-inflammatory cytokine responses, while spleen involved pro-inflammatory cytokine and activated T-cells responses. This study highlighted varied local host immune profiles among target organs during systemic *C. albicans* infection, which could be of importance for future work in designing targeted immunotherapy through immunomodulatory approaches.

Modifications in translation initiation region enhance Cry2A expression in *Bacillus* host

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Translation initiation is main pillar of protein expression and often referred as rate limiting step which facilitates stable attachment of a ribosome to the mRNA to form translation initiation complex**.** Here we report a method for improving translational initiation efficiency of *cry2Ac11* gene in *Bacillus* host. The *cry2Ac11* gene used as model gene as its low expression was obtained without chaperone. The gene *cry2Aa* are positioned at third position (Orf3) in operon comprising orf1, orf2, and orf3. It needs accessory protein (ORF2) for crystal formation and high yield. Operon of *cry2Ac11* as well as ORF‟s was cloned in pHT3101 shuttle vector under control of dual promoter cyt1A-p/STAB-SD. Genetic manipulation of *cry2Ac11* gene without helper protein was carried out by optimizing ribosomal binding site and spacer region (RBS-ATG) in translation initiation region (TIR). Mutants were expressed in acrystalliferous *Bt* strain. Higher transcript and protein expression level of mutant *cry2Ac11* was obtained in which triple start codons AUG was introduced in RBS-AUG region. The mechanism of multiple starts codons on hyper expression of recombinant protein without chaperone in prokaryotic expression system still need to investigate and correlate with eukaryotic system. Gene size in transgenic plants can be considered as limiting factor in expression of *cry* toxin, and so this study may be going to solve this problem.

Helix 5678 of GSTM2-2C terminal domain inhibits the cardiac ryanodine receptor by binding to a 22 amino acid fragment in the DR3 region

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Cardiac ryanodine receptor (RyR2) is essential for the release of calcium from the sarcoplasmic reticulum and contraction of heart muscle. Glutathione transferases are endogenous antioxidant enzymes that conjugate the tripeptide reduced glutathione to various toxins. Our previous studies have proven that C-terminal domain of the glutathione transferase M2 (GSTM2-2C) has an isoform specific inhibitory effect on the RyR2 activity. GSTM2-2C was proven to have a higher affinity for the divergent region 3 of the RyR2 (RyR2D3). Objective of this study was to identify the minimum fragment of the C-terminal domain of GSTM2-2 that binds to the smallest fragment of DR3 region of the ryanodine receptor to inhibit the activity of the RyR2. Different helices of the GSTM2-2 C, H5678, H56 and H78, were expressed and purified. The RyR2D3, RyRD3 mutant (G1885E, G1886S) and RyRD3 short peptide were synthesized. Intrinsic tryptophan fluorescence experiments were performed by placing the fragments of GSTM2-2 C in a cuvette and titrating different fragments of the RyR2. They were excited at 280 nm and the fluorescence emission was monitored at 340 nm. Non-linear regression curve was generated and the equilibrium dissociation constants of the reaction between the fragments of GSTM2-2 C and fragments of RyR2 were determined. RyR2D3, RyR2D3 mutant and RyRD3 short peptide gave a Kd (μM) of 57.8 ± 4.2, 43.4 ± 0.8 and 30.2 ± 5.8 respectively. When compared, the most active fragment RyRD3 short gave binding affinities of 134.3 ± 44.7 and 41.2 ± 7.1 μM respectively with H56 and H78. These results were compatible with the results obtained for single channel and Ca^{2+} release experiments. Strongest binding was achieved by the shortest 22 amino acid fragment, RyRD3 short, against helix 5678. Interestingly, the double mutant associated with arrhythmogenic right ventricular cardiomyopathy (ARVC) increased the binding affinity of H5678 to RyR2D3. It is interesting to speculate that H5678 can be developed for the treatment of ARVC which cause sudden cardiac death due to stress induced ventricular arrhythmias. In conclusion, the 22 amino acid region of RyR2 would be an ideal drug target for isoform specific drugs to modulate cardiac RyR2 activity.

Key words: cardiac ryanodine receptor, GSTM2-2, divergent region 3, cardiac arrhythmia, heart muscle

Molecular insight into sulfate assimilation in durian fruits revealed by the integration of metabolome and transcriptome analyses

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Durian (*Durio zibethinus* M.) is an important fruit crop in Thailand as well as in Southeast Asia. It actively produces the infamous sulfur-containing odor during ripening. Sulfate assimilation in plants is very important for the sulfur cycle in many organisms. How sulfate is transported from roots to shoots has been extensively studied. However, sulfate assimilation in a fruit remains elusive. In this study, the integration of metabolomics using CE-TOF/MS and transcriptomics using de novo RNA-seq assembly was performed to reveal metabolic changes involved in sulfur metabolism in durian fruit. Metabolome and transcriptome data validated by real-time RT-qPCR analysis showed that the expression level of genes involved in sulfur metabolism is concurred with the reduced sulfate ion content in placenta and the up-accumulation of sulfur metabolism-related metabolites in pulps, suggesting that sulfate was assimilated into organic compounds during fruit ripening. Remarkably, the high level of γ-glutamylcysteine was found at unripe stage implying that this metabolite might act as a sulfur storage form in the fruit. Using these data, we proposed a model showing sulfur assimilation in the durian fruits. Our findings reveal the understanding of sulfur assimilation in the fruit and further provide basic information to assist durian fruits quality improvements through molecular breeding.

Host-cell metabolism regulates *Toxoplasma gondii* bradyzoite differentiation in skeletal muscle cells

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Developmental switching of *Toxoplasma gondii* from its fast replicating tachyzoite to slow replicating bradyzoite stage preferentially occurs in skeletal muscle and brain. Interestingly, terminally differentiated skeletal muscle cells (SkMCs), i.e. myotubes but not proliferating myoblasts support bradyzoite differentiation. However, the factors that drive this stage conversion of *T. gondii* in myotubes remained to be resolved. By analyzing the host cell metabolome, we have identified candidates that might regulate bradyzoite differentiation in SkMCs. GC-MS analyses of uninfected and *T. gondii*-infected myotubes and myoblasts after labeling with13C-glucose indicated an increased pentose phosphate pathway (PPP) activity in myoblasts whereas myotubes preferentially fueled the tricarboxylic acid (TCA) cycle by anaplerotic reactions. Inhibition of the PPP using the glucose-6-phosphate dehydrogenase inhibitor dehydroepiandrosterone upregulated *T. gondii* bradyzoite antigen (BAG) 1 expression in both cell types. In contrast, inhibiting anaplerosis of the TCA cycle by the pyruvate carboxylase inhibitor phenylacetic acid only slightly downregulated BAG1 expression in myoblasts. Addition of the TCA cycle intermediate analogue dimethyl-α-ketogluterate accelerated BAG1 expression in myoblast but not in myotubes. Furthermore, lower PPP activity as observed in myotubes led to reduced levels of NADPH and higher $NADP^+$/NADPH ratios in myotubes than in myoblasts. Consistent with lower NADPH levels in myotubes, reactive oxygen species (ROS) levels were significantly higher in myotubes than in myoblasts. Modulation of endogenous ROS using the antioxidant N-acetyl cysteine inhibited *T. gondii* bradyzoite differentiation in both myotubes and myoblasts. Interestingly, inducing ROS in myotubes and myoblasts using the oxidants luperox or H_2O_2 accelerated *T. gondii* bradyzoite differentiation in both cell types. Thus, physiological concentration of endogenous ROS as observed in myotubes but not myoblasts might favor *T. gondii* stage conversion in myotubes. Together, these data confirmed that lower PPP activities, higher TCA cycle activities and physiological concentrations of endogenous ROS as observed in mature myotubes can regulate *T. gondii* bradyzoite formation in SkMCs.

Oral Presentations

Session IV

17 August (Saturday), 11.00am-12.30pm

Purification and biochemical characterization of garlic phytocystatin: Insight into the structural and functional alteration of garlic phytocystatin induced by denaturants and pesticides

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Phytocystatins are cysteine proteinase inhibitors present in plants. Cysteine proteinase inhibitors play an essential role in maintaining the proper functioning of all living cells by virtue of its thiol protease regulatory properties. In the present study a phytocystatin has been isolated from garlic (*Allium sativum*) by a simple two-step process using ammonium sulphate fractionation and gel filtration chromatography on Sephacryl S-100HR with a fold purification of 152.6 and yield 48.9 %. The molecular weight of the purified inhibitor was found to be 12.5 kDa as determined by SDS-PAGE and gel filtration chromatography. The garlic phytocystatin was found to be stable under broad range of pH (6–8) and temperature (30◦C–60◦C). Kinetic studies suggest that garlic phytocystatins are reversible and non-competitive inhibitors having highest affinity for papain followed by ficin and bromelain. Secondary structure analysis was performed using CD and FTIR. Garlic phytocystatin possesses 33.9% alpha-helical content as assessed by CD spectroscopy. Denaturation of garlic phytocystatin has been investigated using urea and guanidine hydrochloride (GdnHCl). The inhibitory activity of garlic phytocystatin decreases with increasing concentration of denaturant. Increased fluorescence intensity along with red shift reflects the unfolding of garlic phytocystatin at higher concentration of denaturant. GdnHCl induced unfolding showed presence of indiscernible intermediates followed by ANS binding studies. However, denaturation by urea did not show any intermediates. Mid-point transition was observed at 4.7 ± 0.1 M urea and 2.32 ± 0.1 M GdnHCl. Circular dichroism and FTIR results indicate the 50 % loss of secondary structure at 5 M urea and 2.5 M GdnHCl. Carbendazim is a broad spectrum benzimidazole fungicide while oxyfluorfen is a nitrophenyl ether herbicide which is used to ensure plants' protection from pest and pathogens' invasion. Interaction of carbendazim and oxyfluorfen with garlic phytocystatin has been investigated through various biophysical which showed binding between them with consequent modulatory effects. The anti-papain inhibitory assay suggests that incubation of garlic phytocystatin with carbendazim and oxyfluorfen disrupts its inhibitory activity. UV spectroscopy confirmed the formation of garlic phytocystatin and pesticide complex. Intrinsic fluorescence suggests binding of carbendazim and oxyfluorfen to garlic. Synchronous and 3-dimensional fluorescence spectroscopy also revealed alteration in the microenvironment around aromatic residues. Secondary structure analysis confirmed that binding of carbendazim and oxyfluorfen decreases the alpha-helical content of garlic phytocystatin. Collectively, these results demonstrated that garlic phytocystatin exhibited significant structural and functional alteration upon interaction with carbendazim and oxyfluorfen. Since, garlic phytocystatin is involved in various regulatory processes, therefore, its structural or functional alteration may lead to disruption of physiological and biological balance within the plant. Hence, our study signifies that exposure of denaturants and pesticide to plant exerts physicochemical stress within the plant.

Identification of a role for Rab11 in endosomal-lysosomal system homeostasis using CRISPR/Cas9

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The trans-Golgi network (TGN) and the recycling endosomes constitute two major intracellular sorting hubs which are spatially close to each other and are extensively involved in both the anterograde and retrograde trafficking pathways. There is considerable membrane flux between the two compartments to account for the flow of cargo which also influences the dynamics of the endosomal-lysosomal system. The maintenance of the organelle identities as well as spatial positioning of the individual organelles are mediated through recruitment of cytosolic proteins such as Rab proteins. Rab11a and Rab11b are small GTPases which localize to the recycling endosomes and early endosomes. However, the role of these Rabs on endosomal biology has not been completely defined. Stable HeLa cells devoid of either Rab11a or Rab11b were generated using the CRISPR/Cas9 system to functionally dissect the roles of these two Rab11 family members in the recycling and endosomal-lysosomal system. Both Rab11a and Rab11b were required to maintain the morphology of the recycling endosomes, however only the deletion of Rab11a had a significant effect on the recycling of transferrin receptor. Deletion of either Rab11a or Rab11b resulted in the formation of enlarged early endosomes, leading to a perturbation in the endosomal-lysosomal maturation pathway in Rab11a knockout cells with an associated functional defect in intracellular recycling of the cation-independent mannose 6-phosphate receptor between the late endosomes and the TGN. Overall, these results indicate that Rab11a and Rab11b have some overlap in function however there are some clear functional differences associated with the biology of endosomal-lysosomal maturation.

Physical coherence and network analysis reveals NEDD4 and STAMBP as novel regulator of exosomes biogenesis in colorectal cancer cells

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Exosomes are small membrane extracellular vesicles that are secreted under pathophysiological conditions. It contains biologically active cargos that stimulate downstream signalling in the recipient cells. The immense biological functions of exosomes urge the necessity to deepen the understanding of exosomes secretion and cargo packaging. Here, we study the novel regulators of exosomes biogenesis using an integrated approach of physical coherence and network-based analysis. Using this model, we indented to identify previously unrecorded regulators of exosome secretion in colorectal cancer (CRC) cells. We identified 193 novel regulators of exosomes secretion with a significant enrichment of ubiquitin-specific proteins such as NEDD4 and STAMBP. Next, the role of NEDD4 and STAMBP in exosomes biogenesis was evaluated by molecular biology and biochemical experiments. To address this, CRISPR based knockout (KO) of NEDD4 and STAMBP was generated in LIM1215 colorectal cancer cells. We show that loss of NEDD4 impairs exosome production hence is a positive regulator of exosomes secretion while loss of STAMBP results in increased exosome secretion. Our study also confirms that loss of NEDD4 results in less Alix ubiquitination and enhances the fusion of MVBs with lysosomes. Accordingly, inhibition of autophagy rescues the release of exosomes in KO cells. These results identify NEDD4 as a novel regulator that controls exosomes secretion in CRC cells.

Keywords: exosomes, physical coherence, biogenesis, cancer

Programmable DNA looping using engineered bivalent dCas9 complexes

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DNA looping is a ubiquitous and critical feature of gene regulation. Although DNA looping can now be efficiently detected, tools to readily manipulate DNA looping are lacking. Here, we report the development and use of a set of CRISPR-based DNA looping reagents for the creation of programmable DNA loops. DNA cleavage-defective Cas9 proteins of different specificity were linked by heterodimerization or translational fusion to create bivalent complexes able to link two separate regions of DNA. A statistical mechanical model was developed to describe bivalent dCas9-mediated DNA looping and modelling-assisted experimental design was used to establish the key determinants for maximal looping efficiency. After model-directed optimization, the reagents were validated using a quantitative DNA looping assay in *E. coli* cells. Overall looping efficiency could be significantly improved by loop multiplexing. As a proof-of-principle, the bivalent complexes were used to activate an inserted reporter gene by rewiring *E. coli* chromosomal DNA to bring a distal enhancer element, located ~12kb away, to the gene promoter. Such reagents should allow manipulation of DNA looping in a variety of cell types, aiding understanding of endogenous loops and enabling the creation of new regulatory connections.

Keywords: DNA looping, protein engineering, dCas9, NtrC, *E. coli*

Improvement of maltogenic amylase transglycosylation activity by reaction equilibrium control and protein engineering approach

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Maltogenic amylase is an enzyme that performs hydrolysis and transglycosylation at the same active site by breaking and forming, respectively, α-(1,4)-glycosidic linkages of carbohydrate molecules. This valuable enzyme is a great producer of malto-oligosacharides as potential prebiotic and functional food ingredients. However, production of malto-oligosacharides by the enzyme is always limited by its competing hydrolyzing activity. This study reported enhanced transglycosylation activity of maltogenic amylase (MAG1) from *Bacillus lehensis* G1 by two approaches; reaction equilibrium control and protein engineering. Reaction conditions such as time, temperature, enzyme loading and substrate loading were manipulated and malto-oligosacharides production pattern was recorded. MAG1 structure was constructed using homology modeling and docking, then rational design was performed to construct mutant enzymes with predicted higher transglycosylation activities. Mutations were constructed using site-directed mutagenesis and transglycosylation activity was tested for each mutant. Findings from the study revealed that hydrolysis and transglycosylation activity levels were greatly shifted at various reaction conditions. The highest yield of 38 % malto-oligosaccharides was obtained from the optimum condition for transglycosylation with the presence of maltooligosaccharides longer than maltoheptaose. In addition, mutants with low steric interference effect, increase in active site flexibility and hydrophobicity improved the transglycosylation to hydrolysis ratio up to 4.0-folds, leading to improved production of malto-oligosaccharides with higher degree of polymerization. The strategies presented here successfully improved the transglycosylation activity of MAG1 to produce malto-oligosaccharides as potential prebiotics and functional food ingredients.

Keywords: maltogenic amylase, malto-oligosaccharides, transglycosylation, hydrolysis, protein engineering

OAS1 and OAS3 act as negative regulators of the expression of chemokines and interferon-responsive genes in human macrophages

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Upon viral infection, the 2', 5'-oligoadenylate synthetase (OAS)-ribonuclease L (RNaseL) system works to cleave viral RNA, thereby blocking viral replication. However, it is unclear whether OAS proteins have a role in regulating gene expression. Here, we show that OAS1 and OAS3 act as negative regulators of the expression of chemokines and interferon-responsive genes in human macrophages. Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein-9 nuclease (Cas9) technology was used to engineer human myeloid cell lines in which the OAS1 or OAS3 gene was deleted. Neither OAS1 nor OAS3 was exclusively responsible for the degradation of rRNA in macrophages stimulated with poly(I:C), a synthetic surrogate for viral double-stranded (ds)RNA. A mRNA sequencing analysis revealed that genes related to type I interferon signaling and chemokine activity were increased in OAS1$^{-/-}$ and OAS3$^{-/-}$ macrophages treated with intracellular poly(I:C). Indeed, retinoic acid-inducible gene (RIG)-I- and interferon-induced helicase C domain-containing protein (IFIH1, or MDA5)-mediated induction of chemokines and interferon-stimulated genes was regulated by OAS3, but Toll-like receptor 3 (TLR3)- and TLR4-mediated induction of those genes was modulated by OAS1 in macrophages. However, stimulation of these cells with type I interferons had no effect on OAS1- or OAS3-mediated chemokine secretion. These data suggest that OAS1 and OAS3 negatively regulate the expression of chemokines and interferon-responsive genes in human macrophages.

Early blockade of C5a is not robustly recuperative in systemic inflammatory responses after polytrauma

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Recent evidences have pointed towards the exacerbation of systemic inflammatory responses when blood loss (hemorrhagic shock) accompanies multiple injuries (or polytrauma). Polytrauma is one of the topmost contributors in critical care unit mortalities, additionally posing to be a diabolic challenge for clinicians to deal with. As the response of the immune system is often a unanimous cause contributing to the complex pathophysiology of such patients, detailed understanding of the same is the need of the hour. After defining the systemic changes observed in murine model of polytrauma (PT) combined with hemorrhagic shock (HS) and PT+HS patients, our group was interested to see if blockade of complement anaphylatoxin C5a is protective in this context. The usage of a novel system to block C5a activity with a spiegelmer, it was shown that survival and organ damage could be improved in a cecal ligation puncture model of murine sepsis. We first tested the efficacy of the spiegelmer NoxD21, a PEGylated aptamer targeting C5a activity, in *in vitro* studies with human neutrophils and found it to obliterate effects like neutrophil chemotaxis, elastase secretion and glucuronidase secretion. Subsequently, we treated our PT+HS mouse model, where a combined traumatic brain injury, lung chest trauma and femoral fracture was coupled to controlled hemorrhagic shock, with NoxD21. 8-12 week old male C57BI6 mice were assigned into 4 groups, Sham + Control (Ctrl), Sham + NoxD21, PTHS + Ctrl and PTHS + NoxD21. However, the treatment with the aptamer did not alter systemic inflammatory cytokine levels, like IL-6, TNF-alpha and KC. So as to confirm, if C5a has a unilateral control over enunciating inflammatory responses post polytrauma, we used C5a receptor 1 and receptor 2 double knockout mice (C5aR1/2 KO) and C5aR2 KO mice to test if the absence of C5a downstream responses rescues PTHS mice. Inflammatory cytokine levels and organ damage markers were tested in plasma and bronchoalveolar lavage fluid (BAL). While IL-6 was significantly reduced in BAL from the C5aR1/2KO PTHS mice as compared to the WT PTHS mice, the overall inflammatory cytokine profile and organ damage was not affected due to C5aR deficiency. We thus concluded that, C5a blockade cannot rescue proinflammatory responses early after PTHS.

Oral Presentations

Session V

17 August (Saturday), 2.30-5.00pm

A "needleless" approach to detect HPV-driven oropharyngeal cancer

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There has been a significant rise in the incidence of oropharyngeal cancer (OPC) associated with high-risk human papillomavirus (HPV), predominantly HPV-16 infections in high-income countries, especially when compared to HPV-negative head and neck cancer (HNC). Growing evidence supports the concept that exosomes (30 –150 nm) loaded with unique bio-components (DNA, RNA and protein) play a salient role in cancer development and progression. However, the role of exosomes in saliva obtained from HPV-driven OPC is still far from clear. Morphology and molecular features of exosomes derived from three different saliva sampling methods: unstimulated saliva, acid-stimulated saliva and salivary oral rinses were examined using Transmission electron microscopy (TEM), nanoparticle tracking (NTA) and western blot analysis. HPV-16 DNA detection in salivary exosome was determined using qPCR method. Proteome profile of salivary exosomes derived from both cancer-free controls and HPV-driven OPC patients was characterized using liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-MS/MS). Here, we show that the unstimulated saliva had the greater abundance of exosomes when compared to the other sampling methods. In fact, the three common exosome markers (CD9, CD63 and CD81) were higher in unstimulated saliva method. Nevertheless, no appreciable difference in exosome morphology was found among the three different sampling methods. Furthermore, only salivary exosome derived from HPV-driven OPC patients had a detectable level of HPV-16 DNA. Intriguingly, the proteomic signature of salivary exosome was significantly different between cancer-free controls and HPV-driven OPC. Taken together, our results showed that unstimulated saliva is an optimum sampling method for exosome characterization. More importantly, the development of a low cost non-invasive saliva-based test using both salivary exosomal DNA and protein will offer an opportunity to detect HPV-driven OPC, thereby opening new avenues in the future for clinical and commercial translation.

Keywords: human papillomavirus, oropharyngeal cancer, saliva, exosome, biomarker

Increased JNK signaling occurs in accelerated liver tumour development in obese and diabetic mice

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Obesity and diabetes are independent risk factors for hepatocellular carcinoma (HCC). However, the contribution of each metabolic condition to hepatocarcinogenesis has not been clarified. Earlier, we demonstrated that obese diabetic *foz/foz* mice exhibit accelerated diethylnitrosamine (DEN)-induced HCC [1]. To establish whether obesity itself or diabetes is more relevant to enhanced HCC development, we compared chemically induced HCC between diabetic *Alms1* mutant (*foz/foz*) NOD.B10 and non-diabetic *foz/foz* BALB/c mice. Male *foz/foz* and *Wt* NOD.B10 and BALB/c mice were injected with DEN (10 mg/kg) at 12-15 days, controls with saline. Hepatic protein expression was assayed by immunoblotting and immunohistochemistry. Both strains of *foz/foz* mice developed equivalent obesity, but metabolic complications of obesity, including hepatomegaly, insulin resistance with hyperinsulinemia, and hyperglycemia, occurred only in *foz/foz* NOD.B10 mice. At 6 mths, the incidence of liver tumours was significantly higher in *foz/foz* NOD.B10 than *foz/foz* BALB/c mice (100 % vs 40 %). Liver nodules were also more numerous and larger in *foz/foz* NOD.B10 compared with BALB/c counterparts. Increased liver injury evident by serum ALT and hepatic Bax expression occurred in diabetic obese NOD.B10 but not in non-diabetic obese BALB/c mice. Hepatocyte proliferation (cyclin D1 and PCNA) was comparable between the two obese lines. Despite the obvious difference in levels of serum insulin in response to obesity, insulin-mediated mammalian target of rapamycin (mTORC1) activation was similar between groups. Instead, activation of c-Jun N-terminal kinase (JNK) signalling was clearly enhanced in *foz/foz* NOD.B10 mice but unaltered in *foz/foz* BALB/c mice. This was associated with the up-regulation of c-Myc. There was a parallel increase in activation of nuclear factor E2-related factor 2 (NRF2) in *foz/foz* NOD.B10 vs *foz/foz* BALB/c mice, inferring enhanced oxidative stress in obese mice with diabetes. Diabetes accelerates liver tumour development in obese mice. The pathogenic mechanisms may include increased liver injury related to metabolic obesity and oxidative stress-induced JNK activation.

[1] E Arfianti, CZ Larter, SS Lee, V Barn, G Haigh, MM Yeh, GN Ioannou, NC Teoh, GC Farrell. (2016). Obesity and diabetes accelerate hepatocarcinogenesis via hepatocyte proliferation independent of NF-kB or Akt/mTORC1. *J Clin Transl Res*; 2(1):1

Rutin ameliorates sodium fluoride induced hypertension and cardiorenal dysfunction via Kim-1/NF-κB/Nrf2 signaling pathways

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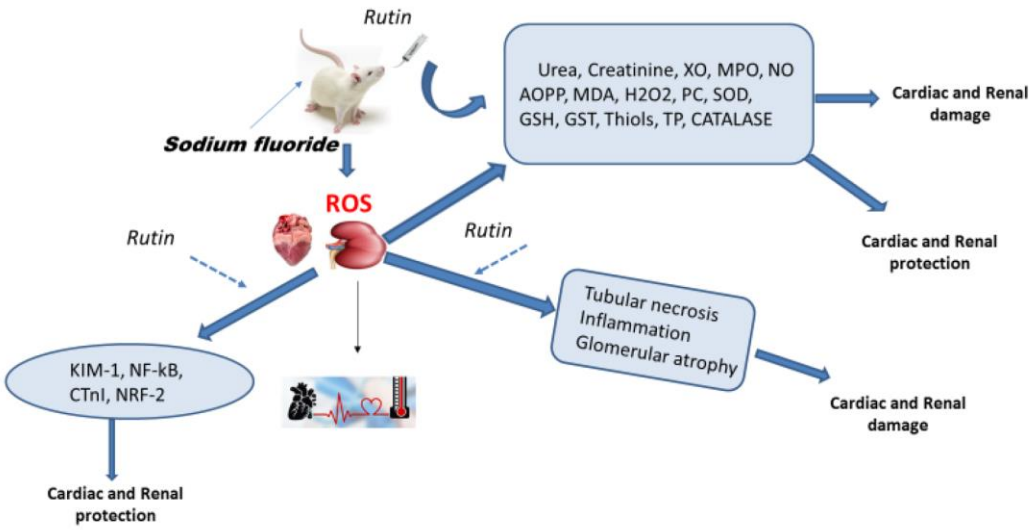
Sodium fluoride is one of the neglected environmental contaminants. Inorganic fluorides in the environment are found in the air, water, and land. In the study, forty-male Wistar albino rats were randomly divided into four groups with 10 rats in a group. Group A was the control group which was given normal saline, Group B was exposed to 300 ppm of NaF in drinking water, while Groups C and D received NaF along rutin (100 mg/kg and 200 mg/kg) orally daily for a week. Administration of NaF alone led to significant increases in blood pressure, elevations of markers of oxidative stress, depletions of antioxidant and deceased serum nitric oxide. Immunohistochemistry revealed higher expressions of kidney injury molecule I (Kim-1), nuclear factor kappa beta (NF-κB), and down regulation of nuclear factor erythroid 2related factor 2 (Nrf2) in rats administered NaF. Together, rutin co-treatment with NaF normalized blood pressure, ameliorated cardiac and renal markers of oxidative stress, improved antioxidant status, lowered Kim-1 and NF-κB expressions, and improved nitric oxide bioavailability.

Keywords: antioxidant, hypertension, oxidative stress, rutin, sodium fluoride



Purification of human 6 phosphogluconate dehydrogenase (h6PGD) from *Escherichia coli* and development of 6PGD enzymatic activity assay

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Reprogrammed tumor metabolism unitedly drive malignant behaviour of cancer cells by providing not only energy (ATP) but structural units for macromolecule synthesis (amino acids, nucleotides and sugars), redox regulators (NADPH), and reducing powers. The pentose phosphate pathway (PPP) is a major pathway for energy metabolism. Currently, there is rising contemplation on oxidative pentose phosphate pathway (PPP) enzymes as potential therapeutic hits due to their affiliation with tumor metabolism. The 6-Phosphogluconate dehydrogenase (6PGD) is the third enzyme involved in pentose phosphate pathway (PPP). This enzyme generates ribulose-5-phosphate with the reduction of $NADP^+$ to NADPH. 6PGD was recently reported as a novel drug target in various cancers. During this study, firstly, we cloned this gene in expression vector, pET28a. Next we established an expression and purification scheme of recombinant enzyme human 6PGD from *Escherichia coli.* We have successfully constructed the plasmid and then protein was purified by Ni-NTA bead and quality of protein was 95 % pure that was checked by SDS-PAGE. This purified protein is used for screening. By using this purified recombinant 6PGD protein, we devised the colorimetric assay that was suitable for high-through put screening for human 6PGD inhibitors. To recognize small molecules 6PGD enzymatic inhibitors, the chemical array-based screening was accomplished through using the purified 6PGD protein. We attained 160/29707 hits of 6PGD. For verification of these hits and to create IC50 values, second screen with enzymatic activity assays were performed, 5/160 hits of 6PGD were confirmed. We have anticipated that enzymatic assay-based screening supported the new unbiased approaches to support the identification and characterization of novel drug candidates that counteracted drug resistance and with maximal clinical efficacy.

Neutralization capacity of Thai green pit viper antivenom to venom of Myanmar green pit viper

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Green pit viper (*Trimeresurus* spp) bite occurred throughout Myanmar and contributed the second most cases after Russell's viper bites in a prospective study in Mandalay during 2016-2017. Massive local swelling, blisters, necrosis and bleeding from the wound are seen in severe case. Development of coagulopathy would lead to shock in patients. Antivenom for Myanmar green pit viper is not available and most of patients were treated with Russell's viper antivenom because of prolonged clotting time was observed from both species. Thai green pit viper antivenom raised to *T. albolabris* was found to be effective against five *Trimeresurus* venom in Thailand. The neutralization capacity of Thai green pit viper antivenom raised to *Trimeresurus albolabris* towards the venom from *Trimeresurus erythrurus* in Myanmar has been examined. This is the very first study on venom of Myanmar green pit viper. The sublethal dose (LD50) is 93.76 μg/mouse. The minimal haemorrhagic dose (MHD) is 0.51 μg/mouse. The minimal coagulation dose for plasma is 98 μg/mL and for fibrinogen solution is 140 μg/mL. The antivenom neutralized lethal, haemorrhagic, coagulation activities of the venom. The effective dose (ED50) of Thai green pit viper antivenom on lethality is 1/10 (venom/antivenom ratio). The MDH-medium effective dose (MHD50) is 1/4 (venom/antivenom ratio). The minimum volume of antivenom which completely prevents clotting is 30 μL (MCD-P100) and 51 μL (MCD-F100) for plasma and fibrinogen solution respectively. The neutralization effect is not seen up to 4 mL of Myanmar Russell's viper antivenom when testing against MCD-P dose. In conclusion, Thai green pit viper antivenom will be the better choice for patients with Myanmar green pit viper bite, rather than using Myanmar Russell's viper antivenom before production of specific Myanmar green pit viper antivenom. These findings will highlight the need and provide basic data for development of specific Myanmar green pit viper antivenom.

Key words: Myanmar green pit viper venom, Thai green pit viper antivenom, neutralization

Effects of *Mif* deficiency and fructose-enriched diet on lipid metabolism in the mouse liver

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The macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine involved in metabolic inflammation and regulation of energy metabolism in the liver. Genetic deletion of *Mif* may contribute to the development of systemic insulin resistance, while fructose overload can disturb hepatic lipid metabolism leading to steatosis, inflammation and type 2 diabetes. The aim of the present study was to elucidate the impact of combined effects of *Mif* deficiency and fructose-enriched diet on insulin sensitivity and lipid metabolism in the liver of male mice. We analysed the effects of 9-week 20 % fructose-enriched diet on indicators of systemic insulin sensitivity, liver histology and biochemical parameters of lipid metabolism in wild type and MIF deficient ($MIF^{-/-}$) C57Bl/6J mice. The expression of the following lipogenic genes was examined: fatty acid synthase (*Fas*), acetyl-CoA carboxylase (*Acc*) and stearoyl-CoA desaturase-1 (*Scd1*). Levels of insulin-regulated transcriptional factors involved in lipogenesis (sterol regulatory element-binding protein-1c, SREBP-1c and carbohydrate-response element-binding protein, ChREBP), together with the expression of hepatic fatty acid metabolism regulator (peroxisome-proliferator-activated receptor α, PPARα) were also analysed. *Mif* deficiency did not affect plasma free fatty acid and triglyceride levels, but impaired systemic insulin sensitivity regardless of the diet. In $MIF^{-/-}$ animals, liver histological analysis confirmed the presence of lipid droplets and focal necrosis, but these effects were more pronounced in $MIF^{-/-}$ mice on fructose diet. Although *Acc* and *Fas* levels were unchanged, elevated levels of *Scd1*, SREBP-1c and ChREBP, together with decreased PPARα protein level, were most likely responsible for the lipid accumulation observed in the liver of $MIF^{-/-}$ animals. In conclusion, the results show that energy-rich fructose diet potentiates the effects of *Mif* deficiency on development of fatty liver and systemic insulin resistance.

Keywords: *Mif* deficiency, fructose-enriched diet, liver, lipid metabolism, insulin resistance

Interaction of p130 protein with HPV 16 and 18 E7 oncoproteins

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p130 is a member of the pocket protein family involved in cell cycle regulation. It mainly functions as an oncosuppressor by interacting with the E2F4 and E2F5 transcription factors. Indeed, p130 has been found to be altered in various cancer types; it serves as a valuable prognostic marker. High-risk human papillomaviruses (HPVs) types, including 16 and 18, are strongly associated with cervical cancer. Previous studies have demonstrated that HPV E7 protein targets p130 to promote host cell exit from quiescence (G0) state to enter the S phase. With this understanding, we further investigated the mechanism by which E7 is able to cause deregulation of the host cell cycle, alter the localization of p130 and affect the expression of differentiation genes in HPV 16/18 E7-transfected cells. Co-immunoprecipitation, Western blot analysis, immunofluorescence microscopy, flow cytometry and quantitative-PCR were employed to investigate the loss of p130 and its mechanism in HPV 16/18 E7-transfected HaCaT (human keratinocytes) cells. HPV16E7 (CaSki) and HPV18E7 (HeLa)-transformed cells were used to complement the ectopic expressions of E7. Our study found that HaCat cells showed higher level of p130 than HPV-transformed cell lines and we verified with immunofluorescence analysis that the HPV-infected cells and the transformed cells exhibited higher cytoplasmic p130 signal presumably due to E7-mediated p130 degradation in the nucleus and/or E7-mediated p130 nuclear export. We also found a significant increase in S/G2 phase in the HPV-transformed cells as E7 has been shown to stimulate proliferation by deactivating Rb-dependent G1/S checkpoint. Concurrent with the increased proliferation, we observed downregulation of keratinocyte differentiation markers, p130, K10 and involucrin, in the transformed cells. Lastly, we verified that the exported p130 underwent proteasomal degradation, confirming the cellular localization pattern of p130 from nucleus to cytoplasm which is commonly seen in cancerous cell. These findings provide evidence that delocalization of nuclear p130 leads to cell cycle deregulation and also impaired of cell differentiation.

Keywords: p130, HPV E7 proteins, p130 localization, cell cycle

Isolation and assessment of trehalose-6-phosphate synthase and Na^+/H^+ antiporter genes under salinity stress in *V. turcica* (Fabaceae; Papilionoideae)

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Abiotic stress factors in Turkey as well as all over the world seriously affect production. Salt stress, one of the abiotic stress factors, affects the photosynthesis and physiological functions of plants negatively and causes loss of product. Molecular control mechanisms that are effective intolerance to soil salinity and identification of genes involved in these mechanisms constitute the critical field of study in plant science. Trehalose-6-phosphate synthase (TPS) is one of the essential enzyme genes involved in trehalose biosynthesis which is protective against salt stress. Also, the vacuolar Na+/H+ antiporter gene (*NHX*) is known to be useful in salt tolerance. *Vuralia turcica* located in the family Fabaceae. This plant is a critically endangered endemic plant species in Turkey and grows naturally in saline environments. In this study, the *TPS* and *NHX*–like genes in *V. turcica* were partially sequenced using degenerate primers and submitted to the NCBI database (accession numbers MK120983 and MH757417, respectively). The expression levels of the genes encoding *TPS* and *NHX* were investigated. High expression levels of *TPS* and *NHX* genes were determined in plants under high salt stress (2000 ppm NaCl). The findings suggest that *TPS* and *NHX* genes might play a crucial role in the salt tolerance mechanism of *V. turcica*. The current study represents the first study of *TPS* and *NHX* genes in *V. turcica* and provides a starting point for future functional studies of these genes in Fabaceae.

Keywords: isolation, *NHX*, salt tolerance, *TPS*, *Vuralia turcica*

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