S1-0001

Patterns of Human-Wildlife Conflict and Conservation Implications in the Indian Trans-Himalaya

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Large carnivore-human conflict is a worldwide issue and co-existence rarely exists. We investigated the grass root factors causing large carnivore-human conflict in Kargil, Ladakh and examined efficacy of participatory rural appraisal tools in validating questionnaire surveys and share of livestock in the wild predator's diet. Very few studies have been conducted to understand the patterns of human-wildlife conflict in the high altitude remote, inaccessible and inhospitable terrain of the west Himalaya and implements conservation strategies. Globally threatened snow leopard (*Panthera uncia*) and more common wolf (*Canis lupus*) were the two main wild predators in Kargil. About 8.3% livestock loss (2009-2012) was due to predation by large carnivores, which resulted in strong negative perception (46%) amongst the local communities. A total of 1113 heads of livestock were reportedly killed by wolf (43.6%) followed by unknown predators (31.4%) and snow leopard (21.5%) in the study site, which comes to 2.8% annual livestock losses of the region. Poor livestock husbandry practices and traditional livestock corrals were found to be the major causes contributing in the large carnivore-human conflict in Kargil. Scat analysis also revealed significant amount of livestock in the diet of snow leopard (47%) and wolf (51.2%). Based on the research findings, we worked with the local communities to sensitize them about wildlife conservation and extended limited support for predator proof livestock corrals at a small scale. Eventually it helped in reducing conflict level and conserving the globally threatened carnivores in Kargil. We conclude that the notion that the negative perception of locals towards livestock predation is not solely due to their economic losses but also because Kargil was a neglected region for wildlife research and conservation programmes.

S1-0002

Monitoring Illegal Trade in Snow leopards: 2003-2014

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Illegal trade in snow leopards (*Panthera uncia*) has been identified as one of the major threats to long-term survival of the species in the wild. To quantify severity of the threats to dwindling snow leopard population, we examined market and questionnaire surveys, and information from the published and unpublished literature on illegal trade and poaching of snow leopards. We collected information from 11 of the 12 snow leopard range counties in central and southern Asia, barring Kazakhstan, and reported 439 snow leopards (88 records) in illegal trade during 2003e2014, which represents a loss of approximately 8.4%e10.9% snow leopard population (assuming mid-point population of 5240 to minimum population of 4000 individuals) in a period of 12 years. Our data suggested a 61% decadal increase in snow leopard trade during 2003e2012 compared with 1993e2002, while taking the note of significant strengthening of wildlife enforcement and crime control network in the decades of 2000s and 2010s. We found 50% prosecution rate of snow leopard crimes resulting in only 20% conviction rate

globally. Many limitations e.g., secretive nature of illegal trade, ill developed enforcement mechanism, poor and passive documentation of snow leopards' seizures, restricted us to reflect actual trend of snow leopards' illegal trade. Even on a conservative scale the present situation is alarming and may detrimental to snow leopard conservation. We propose an effective networking of enforcement efforts and coordination among the law enforcement agencies, efficient collection of data and data management, and sharing of intelligence in snow leopard range countries, could be useful in curbing illegal trade in snow leopards in central and southern Asia.

S1-0003

Organity and kingdom periodical table of organisms

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Organity analogous to parity in quantum mechanics is introduced to address the evolution of cell organisms on the Earth. The Earth is in the midst of the sixth extinction, and human is at the mirror point or near the mirror point of the whole history of evolution. Human is both the terminator of the first half and the activator of the second half of the evolution game on the Earth. Before the mirror point, the evolution of cell organisms followed the rule called half-hist-hump, while after the mirror point, the evolution of cell organisms will follow the rule called bime-been-bang. The first several Fibonacci numbers may be followed by the pan-symbiosis events to create prokaryotic cells, eukaryotic cells and multicellular organisms leading to Cambrian explosion and subsequent evolution of terrestrial organisms. The kingdom periodical table of organisms (KPT) corresponding to the periodic table of the chemical elements (PTE) is proposed to arrange all cell organisms in the current dominant 7 kingdoms (Bacteria; Archaea; Protozoa and Animalia; Fungi, Chromista and Plantae; 1+1+2+3, following Fibonacci sequence) and our 12 kingdoms (1+1+2+3+5, also following Fibonacci sequence) proposed here, respectively. The kingdom-level classification of life is still widely employed as a useful way of grouping organisms. Here three taxonomic ranks above domain are introduced. For cell organisms, a superspace contains one space, two galaxies and three domains. A domain contains one or more kingdoms. The twelve kingdoms are Probacteria and Bacteria (corresponding to Period 2 in PTE), Proarchaea and Archaea (Period 3), Excavata, Protozoa, Amoebozoa, Animalia, Fungi, Rhizaria, Chromista and Plantae (Period 4). Fungi (Groups IA-IIA) may be more closely related to pan-plants (Group IIIA-VIIA) than to pan-animals (Groups IB-VIIIB) in phylogeny and ecology. Kingdoms Probacteria and Proarchaea in the Galaxy Prokaryota may have been extinct.

S2-0001

RNA interference and Enzymatic Characteristics Analysis of LmCesA1 in Locusta migratoria

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In the long-term use of insecticides, *Locusta migratoria* (Orthoptera: Acridoidea), gradually develops resistance. Carboxylesterases (CarEs) are necessary in metabolism of specific hormones and detoxification of dietary and environmental xenobiotics in insects. The nymph mortalities increased from 34.3 to 65.2% after LmCesA1 was silenced when malathion was applied. We got recombinant protein of LmCesA1 by using Bac-to Bac expression system and purified target protein by affinity chromatography. LmCesA1 had hydrolytic activity of 544.5 nmol·min-1·mg-1 with β-NA as substrate. LmCesA1 showed the highest activity at pH 8.5 and 20℃. Whereas, the enzyme showed no activity at pH 3-6, and lower activity at pH 9-10. It was stable under 10-30 ℃ , but had poor stability above 40 ℃ . The *K*m was 0.143 mmol·L-1 and *V*max was 1.43 mmol·min-1·mg-1 at 37℃ and pH 7.5 . These results further confirmed that LmCesA1 may be involved in detoxification of malathion in *L. migratoria*.

S6-0001

Effect of recombinant follicle stimulating hormone (rFSH) on superovulation in Boer Goat

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Objective: The experiments were to investigate the effect of recombinant follicle stimulating hormone injection (rFSH) on Superovulation in Boer goats. The first experiment examined the effect of different doses of FSH on Superovulation in goats. The second experiment examined whether there were differences in the effect of different batches of rFSH on Superovulation in goats.

Method: Superovulation was performed by CIDR + FSH + PG.

Results: The number of embryos in the first experiment of high dose (rFSH 600IU/goat), middle dose (rFSH 400IU/goat), low dose (rFSH 200IU/goat) and control group (Folltropin-v) was 15.0±2.9, 16.8±3.3，13.0±2.9 and 13.4±2.9. The number of follicles on ovarian surface in each group was 2.2±1.2, 4.0±1.4, 3.2±1.2 and 4.2±1.2. There was no significant difference of in the groups (P>0.05). The same number of embryos could be obtained in the three dose groups. The number of follicles in the three dosage groups was smaller than that in the control group, indicating that the drug dose did not cause ovarian hyperstimulation. The second experiment consisted of 5 groups: A (rFSH 400IU / goat), B (rFSH 400IU / goat), C (rFSH 400IU / goat), Folltropin-v, Ningbo Sansheng FSH.. The results showed that different experiments of drugs had the same effect of superovulation. There was no significant difference in groups (P > 0.05).

Conclusion: The effect of rFSH (200-600 IU) on superovulation of Boer goats was the same as that of Folltropin-v. The effect of different batches of rFSH on superovulation of Boer goats was the same, which indicated that the stability of rFSH in different batches was very good.

S8-0001

Human rhomboid family-1 modulates clathrin coated vesicle-dependent pro-transforming growth factor α membrane trafficking to promote breast cancer progression

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Background: Epidermal growth factor receptor (EGFR) signalling is critical in epithelial cancer development. Human rhomboid family-1 (RHBDF1) facilitates the secretion of TGFα, an EGFR ligand, in breast cancer; however, the underlying mechanism remains unclear. We evaluated the role for RHBDF1 in clathrin-coated vesicle (CCV)-dependent pro-TGFα membrane trafficking in breast cancer cells upon stimulation by G-protein coupled receptor (GPCR) agonists.

Methods: *RHBDF1* was silenced in various breast cancer cells using shRNA. TGFα levels, subcellular localization, and secretion were evaluated using ELISA, immunofluorescent staining, and coimmunoprecipitation. Phosphorylation and expression of relevant proteins were measured by western blotting. RHBDF1-dependent cell viability and invasion were measured.

Findings: RHBDF1 mediates GPCR agonist-induced EGFR phosphorylation by promoting TGFα secretion in various types of breast cancer cells. RHBDF1 not only mediates ADAM17-dependent shedding of TGFα, but is essential in membrane trafficking of pro-TGFα. *RHBDF1* silencing results in blocking of clathrin uncoating from CCV, a crucial step for the plasma membrane release of pro-TGFα. Interaction of RHBDF1 with auxilin-2, a CCV protein, determines the recruitment of HSC70 to CCV to facilitate clathrin uncoating. *RHBDF1* function is required for the proliferation and mobility of breast cancer cells upon stimulation by Sphingosine 1 Phosphate (S1P), a GPCR agonist. We demonstrate a significant correlation between *RHBDF1* overexpression and EGFR activation in breast cancer tissues.

Interpretation: RHBDF1 is an indispensable component of the protein trafficking machinery involved in GPCR-mediated EGFR transactivation, and is an attractive therapeutic target for cancer.

S9-0001

Generation and characterization of fibroblast-specific Basigin knockout mice

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Basigin is well-known as an extracellular stimulator of fibroblasts and may confer resistance of apoptosis to fibroblasts *in vitro* under some pathological status, but its exact functions and the underlying mechanisms in fibroblasts remain poorly understood. The systematically *Basigin* gene knockout leads to the perinatal lethality of mice, which limits the delineation of its functions *in vivo*. In this study, we generated a fibroblast-specific *Basigin* knock-out mouse model and demonstrated the successful deletion of *Basigin* in fibroblasts. The fibroblast-specific deletion of *Basigin* did not influence the growth, fertility and the general condition of the knockout mice. No obvious differences were found in the size, morphology, and histological structure of the major organs, including heart, liver, spleen, lung and kidney, between the knockout mice and the control mice. The deletion of *Basigin* in fibroblasts did not induce apoptosis in the tissues of the major organs. These results provide the first evidence that the deletion of *Basigin* will not confer apoptosis of fibroblasts under the physiological status *in vivo*, and the fibroblast-specific *Bsg* knock-out mice could be a useful tool for exploring the function of Basigin in the fibroblast biology *in vivo*.

S10-0001

Using Neuromodulation to Disrupt Consolidation of Fear Memories

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Anxiety disorder poses one of the biggest threat to mental health in the world, yet current therapeutics have been mostly ineffective due to issues with relapse, efficacy, and toxicity of drugs. Deep brain stimulation (DBS) is a minimally invasive therapy for treatment-resistant psychiatric disorders including anxiety, despite this, very little is known about the effects of DBS on fear memories. In this study, we used a modified plus-maze discriminative avoidance task to model the interaction between innate fear and conditioned fear. We showed that DBS in the medial prefrontal cortex (mPFC) was able to disrupt consolidation, but not acquisition nor retrieval of fear memories. We validated these results using a standard tone-footshock fear paradigm and showed disruption in both tone and contextual fear memory. We further demonstrated short term, but not long term, changes in both dopaminergic receptor, and the immediate early gene c-fos, expression in the ventral, but not dorsal hippocampus. We further showed that dopamine 2 receptors are involved in these effects. Based on our results, we propose a model, using a conceptual artificial neural network, on how neuromodulation is able to disrupt memory. Overall, our data suggest that mPFC stimulation is able to alter fear memories, and should be further studied as a potential therapeutic means for anxiety disorders.

S12-0001

Bio-Responsive Nanomaterials and Drug Delivery Systems for Cancer Therapy

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Owing to the complexities in biological systems, bio-responsive materials and drug delivery are critical to their efficiencies and accuracies as therapeutic agents. Considerable effort has been directed at research aiming to deliver drugs to specific target sites using bioresponsive systems that react to specific external and internal stimuli, including pH, redox, temperature, light, *etc*. Of these, the pH is of particular importance owing its variations between not only the normal physiological environment and tumour microenvironment but also similar variations at the sub-cellular level. Redox-active nanoceria (cerium oxide nanoparticles) has shown potential in cancer therapy owing to its pH-controlled redox behaviour at the cellular level and its regulation of reactive oxygen species (ROS)1,2. In the present work, nanoceria particles of varying morphologies were synthesized by wet chemical methods3 and its delivery to the cancer cells was enhanced by functionalizing with targeted-design, zwitterionic pH-sensitive charge-switchable polymers4. Additionally, to reduce the environmental impact of processing, materials and polymers synthesis were carried out by utilizing green chemical approaches5. Redox efficiency of nanoceria was measured at different bio-mimicking pH levels. Preliminary results suggest that polymer enhanced the cancer cell uptake of nanoceria and functionalized nanoceria is of great potential to use a nanomedicine.

S12-0002

Nuclear translocation of receptor tyrosine kinase MET is stimulated by ARF and inhibited by positively charged food carbon dots through binding to negatively charged phosphor-Tyrosine and BER/NER DNA damage repair

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Nuclear receptor tyrosine kinases are recently found to be aberrantly upregulated in many types of cancer, but regulation of nRTK remains unclear. We previously showed androgen deprivation therapy induces nMET in castration-resistant prostate cancer. Using Arf deficiency in Pten/Trp53 conditional knockout mice model we identified that nMET signaling requires ARF. Accordingly, aberrant MET/nMET elevation correlates with ARF in human prostate cancer specimens. Mechanistically, ARF elevates nMET through stabilizing MET via binding to MET cytoplasmic domain to mediate chemotherapy drug resistance. Furthermore, inhibition of nMET was observed by CNPs sensitizing cancer cells to MET inhibitor through DNA damage response. The inhibition of phosphorylation by nano-carbon dots was identified through binding to the phosphate group of phosphor-tyrosine via computational calculation and in vitro enzyme assay. In addition, In vivo, we found the c-dots inhibited tumor growth of PC3 prostate cancer cells xenograft model through PARP-dependent DNA repair inhibition. Moreover, c-dots induced cell death was correlated with cell detachment and inhibited by extracellular matrix (ECM). This

is consistent with that in leukemia mouse model which lack ECM in the microenvironment, are not sensitized to the c-dots. Positively charged c-dots also bind to negatively charged both double-stranded and single-stranded DNA to cause both BER and NER mediated DNA damage and repair response. Finally, in combination with c-MET inhibitor, c-dots induced higher levels of γH2AX, with decreased cell survival. Our data suggest that c-dots may be efficient in overcoming drug resistance via DNA damage linked cell death, decreased cell adhesion, and inhibition of MET signaling. Thus we proposed a microenvironment-dependent treatment for solid tumors using food nanoscale particles combined with effective kinase inhibitors to prevent drug resistance in preclinical and clinical studies.

S12-0003

Lipopolysaccharide contributes to bacterial resistance to carbon nanoparticles

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Nanoparticles became one of the powerful and promising tools in Biomedicine. In addition, advances in nanotechnology resulted in the production of new nanoparticles from different origins and with more advantageous features. Recent studies have shown that nanoparticles possess antimicrobial activity. However, antimicrobial drug resistance to antimicrobial and the mechanisms remains unclear. Recently it has been shown that Gram-negative bacteria Escherichia coli resistant to silver nanoparticles by upregulation of flagellin, a flagellum protein. Here we report a novel mechanism of bacterial resistance to vegetables-derived green carbon nanoparticle (CNPs). We first found that CNPs inhibit the growth of antibiotic-resistant strains. In addition, these CNPs interact with and damage DNA causing cell death by genomic DNA instability. Moreover, CNPs can decrease the bacterial transcription and motility by affecting flagella. The mechanisms underlying the effect was found by High-Resolution Atomic Force Microscopy (AFM) and SEM which showed that nanoparticles induced breakage of flagella. However, the mechanism of resistance against CNPs was different. By performing correlation tests between Gram character of bacterial strains and survival rate after CNPs treatment, we found that the effect of CNPs correlated with the membrane structure. To test this hypothesis, bacterial cells were cultured with different membrane components as lipid, glucose, and lipopolysaccharide (LPS). As a result, Gram negative but not positive bacterial strains cultured with LPS, but not lipid, sugar showed resistance to CNPs. Thus, our data suggest that CNPs have antimicrobial activity through membrane components (LPS) and LPS contributes to bacterial resistance to carbon nanoparticles. In addition, CNPs combined with drugs targeting LPS can be a great potential avenue against bacterial infection by affecting cellular membrane, damaging flagella, and reducing bacterial motility.

S12-0004

Date pits nanodots as a novel anti-cancer drug acts through inhibition of peroxidase, ROS levels, DNA damage and pH alteration

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Natural food derived carbon nanoparticles along with bioluminescent properties have a potential application in cancer therapy and imaging, but the mechanisms remain elusive. In the following study, we demonstrated that Date pits derived carbon nanodots (Dates-CD) induced cancer cell death by cell cycle arrest and peroxidase activity inhibition, selective apoptosis (Annexin-V), and double-strand DNA (dsDNA) damage. In addition to pH dependent decrease in reactive oxygen species (ROS) levels, western blot showed an increase in cleaved PARP and Gamma-H2AX proteins after treatment. The competitive binding assay suggested a direct binding of nanoparticles to the minor groove of dsDNA. Moreover, atomic force microscopy (AFM) revealed membrane damage caused by Dates-CDs. According to the proliferation assay, Dates-CDs have significantly higher toxicity to the prostate cancer PC3 cells in comparison with the normal rat kidney NRK cells. Thus, consistent experimental results showed Dates-CDs are effective in the selective killing of cancer cells through cell cycle arrest, acidic inhibition of peroxidase and ROS generation. Therefore, this work provided a better understanding of the detailed mechanisms of low-cost natural food derived carbon green nanoparticles with great potential in cancer therapy.

S12-0005

Nanoparticels Inhibit Bacteria Motility through Flagella Breakage

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Bacteria motility is important to allow bacteria to infect and move towards more favorable condition with essential nutrients. Most bacteria usually move using hair-like extensions called flagellum. Flagella can be several thousand nanometers in length, and approximately 20 nm in width. Recent study has shown that flagellum protein flagellin has resistance for silver nanoparticles (Nature Nanotechnology, 2018). In this work, we aimed to find out the effect of several nanoparticles on movement of transformed Escherichia coli strain using GFP time lapse imaging. Results show that at 0.5 mg/ml concentration of nanoparticle derived from material 1 (confidential due to patent application) bacteria motility decreased by up to 90%, whereas at the same concentration of another nanoparticle derived from material 2 (confidential due to patent application), bacteria movement decreased by approximately 50%. Besides the effect of nanoparticles on bacteria movement, it was found that bacteria can also affect nanoparticles by making them aggregate in 2-3 minutes after their interactions. The mechanisms underlying the effect was found by High-Resolution Atomic Force Microscopy (AFM). AFM with high resolution nanoscale measurements and imaging showed that nanoparticles induced breakage of flagella. Thus, our data suggest that these nanoparticles have a great potential in decreasing bacteria motility, and may be used for anti-bacterial infection and bacteria-host interactions.
nanodots

S12-0006

Carbonate apatite nanoparticles-facilitated delivery of siRNA(s) targeting calcium ion channels kills breast cancer cells

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Specific gene knockdown facilitated by short interfering RNA (siRNA) is a potential approach for suppressing expression of ion channels and transporter proteins to kill breast cancer cells. Overexpression of calcium ion channels, namely TRPM7, ORAI1, ORAI3 and ATP2C1 is seen in a variety of breast cancer subtypes. Since naked siRNA is anionic and prone to nuclease-mediated degradation, it has limited permeability across the cationic cell membrane and short systemic half-life, respectively. Carbonate apatite (CA) nanoparticles were formulated, characterized, loaded with TRPM7, ORAI1, ORAI3 and ATP2C1 siRNAs and delivered into MCF-7 breast cancer cells to selectively knockdown the respective calcium ion channels. Individual knockdown of TRPM7, ATP2C1, ORAI1 and ORAI3 genes showed extremely significant reduction ($p<0.001$) in cell viability of MCF-7 cells. Therefore, CA-siRNA-facilitated gene knockdown in vitro holds a high prospect for deregulating cell proliferation and anti-apoptotic pathways modulated by Ca signalling in breast cancer cells.

S12-0007
Potential of nanoparticles for detection of water pollution

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Water pollution is one of the big problems in environmental protection. Development of pathogenic agents in drinking water could be dangerous to human daily life. Monitoring the water quality in environment is also highly demanded. There are many ways being used to detect pathogens with the usage of biosensors. However, sensitivity is a big concern. Our research proposes screening and application of nanoparticles which are able to enhance the color of reaction, thus visualizing the presence of pathogens by difference in color. According to our results, the effect of selected nanoparticles on reaction between substrate TMB and water samples, was significantly investigated, which shows an increase of nanozyme peroxidase activity up to 50%. The future application of this study can assist in detection of pathogens in water reservoir.

S12-0008
Nanoparticle effect on water channel

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Nowadays nanotechnology is one of the most powerful tools that is being studied for use in anticancer therapy and drug delivery. However, the problem of resistance or non-effectiveness of designed nanoparticles can occur. We introduce innovative anticancer therapy which is based on the usage of nanoparticles alongside aquaporins, integral membrane proteins that provide the transport of water across a cell membrane. In this work, our aim was to determine the role of aquaporin in cancer treatment of

nanoparticle-based therapy. The results showed that nanoparticles enhanced the expression of aquaporins on the membrane of cells. Furthermore, aquaporin overexpression with the combination of certain concentrations of nanoparticles and chemotherapy drug significantly decreased the survival rate of different cancer cells used for the experiment. Moreover, mutation of the aquaporin dysregulates the nanoparticles efficiency in combined targeted treatment. Thus, studying water channel effect by nanoparticles would reveal the mechanisms of the nanoparticles in cancer therapy.

S14-0001

Overexpression of MiR-130b via in utero gene transfer accelerates neuronal migration in embryonic cortex and leads to adult behavioral deficits

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MiR-130b, previously known as an onco-miRNA involved in tumorigenesis, was recently found to be dramatically up-regulated in schizophrenia patients in a state-independent manner, implicating a crucial role as a modulator of complex regulatory networks in brain pathologies. To verify its functional role in neuropsychiatric pathology, we made a first attempt to carry out a temporal profiling of miR-130b coupled with in utero electroporation for comprehensive in vivo phenotypic exploration. We performed TaqMan-based quantitative measurement to characterize miR-130b expression pattern in cerebral cortex of normal mice and two established schizophrenia mouse models at various developmental stages. MiR-130b level in mouse embryos was then manipulated via in utero electroporation for subsequent neuronal migration analysis and a spectrum of schizophrenia-related behavioral tests. MiR-130b was progressively increased as brain developed throughout the embryonic stage and gradually decreased after birth. Further comparative analysis in poly I:C prenatal immune challenge mice and DISC1-cc transgenic mice, demonstrated that miR-130b was up-regulated evidently in embryonic cortex in schizophrenia, with a timeline concordant with neuronal migration trajectory. Transient overexpression of miR-130b in the pre- and peri-natal stages led to accelerated neuronal migration during embryonic brain development and schizophrenia-like behavioral abnormalities, including greater response in methamphetamine-induced hyperactivity and deficits in prepulse inhibition after puberty, indicating a state of dopaminergic hyperfunction and impaired sensorimotor gating in miR-130b-overexpression mice. Our work presents the distinct expression profile of miR-130b in neurogenesis, and demonstrates a novel role for miR-130b in the stage-specific modulation of cortical development and high brain functions in adulthood.

S14-0002

Comparative Study on the Genes Involved in the Development of the Telencephalon：Insights into the Innovation of the Mammalian Neocortex

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Little is known regarding the mechanism underlying the innovation of the mammalian neocortex and the mosaic structure of the mammalian telencephalon, in which the neocortex is combined with the phylogenetically conserved subpallium. Here we performed comparative genomics analysis to determine whether there are exceptional variations in genes vital for the development of the pallium (Pax6, Tbr-1 and Emx1/2) in mammals compared to non-mammals or to those for the development of subpallium (Dlx2 and Nkx1/2). We found that these genes display no clear variation in chromosomal duplication/loss or synteny, gene sequence or Darwinian selection between over 50 chordate species, except an additional fragment of approximately 20 amino acids in Emx1, and poly-(Ala) 6–7 in Emx2 from all examined

eutherians but no poly-Ala residues in almost all non-mammals. Furthermore, we found that transfection with mouse Emx2 expression plasmids into cultured cells isolated from the embryonic chick telencephalon significantly increases the Reelin expression in contrast to chick Emx1/2 expression plasmids. We also found that the above increasing activity requires a minimum of six residues in the poly-Ala region, which are required to form a helix in the tertiary structure. Given the role of Reelin in regulating cortical lamination and the brains of Emx1/2 mutant mice similar to non-mammalian brains, our study suggests that acquiring these fragments in mammalian Emx1/2 is involved in the innovation of the mammalian neocortex. The lack of the corpus callosum in monotremes and marsupials, like the phenotypes of Emx2 mutant mice, might ascribe to their Emx2 with poly-(Ala)2.

S14-0003

Notch1 plays a role in the sexual differentiation of a song system nucleus (HVC) in the Zebra Finch

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Significant sex differences are found in many animals, but the underlying neural mechanisms regulating sexual differentiation is still an intriguing puzzle.Unlike mammals, there is no sex-determining gene *Sry* and gene dosage compensation effect in songbirds, so which genes take part in sexually dimorphic and how these genes effect during development need to be investigated. HVC is the critical initial song nucleus in songbirds. We recently found *Notch1* gene to be expressed in significantly sex differences in HVC between male and female Zebra finches by suppression subtraction hybridization. EREs estrogen-responsive elements are also found in *Notch1* gene. The roles of *Notch1* in cell proliferation, migration, survival, and differentiation through juxtacrine cell to cell communication and cross-talk with other pathways have been reported in many studies. So we speculated that *Notch1* genes might be involved in the sexual dimorphism of HVC. In order to explore the roles of *Notch1* in the HVC sexual differentiation in the zebra finch, we first constructed *Notch1* interfering and NICD over-expression lentivirus, and then examined their expression in *vivo*. We injected *Notch1* interfering and NICD over-expression lentivirus into the developing HVC at PHD15 in vivo to study how cell proliferation in short-term groups, neuronal differentiation in middle-term groups as well as the neuronal number, song nuclei volumes and adult song organization in long-term groups were changed. Our results showed that *Notch1* gene was involved in inhibiting cell proliferation in the ventricle zone (VZ) and neuronal differentiation in HVC, promoting cell migration to HVC, reducing newborn projection neurons, decreasing song control nuclei volumes and song complexity. Therefore, *Notch1* has a crucial role in HVC sexual differentiation in the zebra finch.

S16-0001

Middle-East Respiratory Syndrome Coronavirus ORF8b protein suppresses IRF3-mediated type I IFN production

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Middle-East Respiratory Syndrome Coronavirus (MERS-CoV) is a novel human coronavirus that cause acute respiratory distress syndrome with a high mortality rate in infected individuals. MERS-CoV was first discovered in Saudi Arabia in 2012. Individuals infected by MERS-CoV present symptoms significantly different to other HCoV infections including HCoV-NL63, HCoV-OC43 and HCoV-229E which typically result in mild common cold. Characterization of MERS-CoV genome reveals the close phylogenetic relationship with SARS-CoV and therefore raising interests in the pathogenicity of MERS-CoV. Our group has shown that Type I IFN production have been inhibited upon ectopic expression of MERS-CoV open reading frame 8b (ORF8b) protein when stimulated by both poly I:C and sendai virus which are known to induce IFNβ production through RNA-sensing pathway. Co-immunoprecipitation experiments show that ORF8b protein inhibit host type I IFN production by specifically binding to HSP70. We showed that HSP70 in turn interact with a RIG-I pathway component, IKKε which is an essential kinase for IRF3 phosphorylation and dimerization. HSP70 was shown to potentiate IKKε activation in over-expression study and that ectopic expression of ORF8b could revert such IKKε-activating effect mediated by HSP70. Ectopic expression of ORF8b also results in diminished level of IRF3 activation which is consistent to our model. Further to that, recombinant mutant virus depleted of ORF8b showed an elevated level of IFNβ expression at mRNA level when compared to WT recombinant virus without significant difference in the viral titer between WT and Δ8b virus. Our studies show the ORF8b protein is a potent IFN antagonist suppressing type I IFN production that may lead to the pathogenic nature of MERS-CoV.

S16-0002

Characterization of influenza A viruses with polymorphism in PB2 residues 701 and 702

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The 701 and 702 positions of influenza PB2 polymerase subunit are previously shown to have roles on host range determination. However, limited polymorphisms at these two residues are identified in natural isolates, thereby limiting the study of their role in the polymerase. In this study, we generated 31 different viable viruses by random mutagenesis at this region, indicating that these positions can tolerate a wide range of amino acids. These mutants demonstrated varying polymerase activities and viral replication rates in mammalian and avian cells. Notably, some mutants displayed enhanced polymerase activity, yet their replication kinetics were comparable to the wild-type virus. Surface electrostatic charge predication on the PB2 structural model revealed that the viral polymerase activity in mammalian cells generally increases as this region becomes more positively charged. One of the mutants (701A/702E) showed much reduced pathogenicity in mice while the others had pathogenicity similar to the wild-type level. Distinct tissue tropisms of the PB2-701/702 mutants were observed in infected chicken embryos. Overall, this

study demonstrates that the PB2-701/702 region has a high degree of sequence plasticity and sequence changes in this region can alter virus phenotypes *in vitro* and *in vivo*.

Influenza,polymerase,mutation

S16-0003

Effects of SFTS Virus on Type II Interferon Signalling

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Severe fever with thrombocytopenia syndrome (SFTS) is known as an emerging infectious disease presented with severe acute fever, leukocytopenia, thrombocytopenia and other symptoms. SFTS virus (SFTSV), the causative agent, is a tick-born phlebovirus which poses a constant threat to public health in China and elsewhere. WHO has ranked SFTSV as one of the most dangerous emerging infectious diseases, in the category which also includes Zika virus. Unlike Zika virus, SFTSV is severely understudied and our understanding on SFTS and SFTSV is still very limited, and neither antiviral drugs nor vaccines are available for SFTSV. Understanding the pathogenesis and immunopathogenesis of SFTSV infection will facilitate the development of new strategies and approaches to combat SFTSV. In this study, we demonstrated that SFTSV NSs protein augments IFN-γ signalling, as shown by the induction of IFN-γ-stimulated genes (ISGs), IP10, IRF1, MCP1, PD-L1 and PD-L2. By screening a panel of SFTSV proteins (NSs, NP, Gc, Gn and L) for potentiation of GAS activation by IFN-γ, we found that mRNA transcript of IRF1 gene, primarily induced by IFN-γ, was upregulated by two proteins, NSs and Gn. Similarly, NSs highly induced mRNA transcripts of IP10 and MCP1 in IFN-γ-treated HEK293 cells. As an increase in STAT1 phosphorylation might account for NSs potentiation of IFN-γ signalling, we analysed the phosphorylation of STAT1 by Western blotting. Surprisingly, phosphorylation of STAT1 at both positions was higher in the presence of IFN-γ, suggesting that NSs acts mainly on IFN-γ-induced STAT1 phosphorylation. Collectively, our work derives better understanding on the mechanism by which SFTSV usurps IFN-γ signalling to achieve immunosuppression, which will guide the development of new preventive and therapeutic measures.

S16-0004

PacBio But Not Illumina Technology Can Achieve Fast, Accurate and Complete Closure of the High GC, Complex Burkholderia pseudomallei Two-Chromosome Genome

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Although PacBio third-generation sequencers have improved the read lengths of genome sequencing, no study has reported success in using PacBio data alone to completely sequence a two-chromosome bacterial genome from a single library in a single run. In this study, we compared the robustness of PacBio RS II, using one SMRT cell and the latest P6-C4 chemistry, with Illumina HiSeq 1500 in sequencing the genome of *Burkholderia pseudomallei*, a bacterium which contains two large circular chromosomes, very high G+C content, highly repetitive regions and substantial genomic diversity, and represents one of the largest and most complex bacterial genomes sequenced, using a reference genome generated by hybrid assembly PacBio and Illumina datasets with subsequent manual validation. Results showed that PacBio data with *de novo* assembly, but not Illumina, was able to completely sequence the *B. pseudomallei* genome without any gaps or mis-assemblies. The two large contigs of the PacBio assembly aligned unambiguously to the reference genome, sharing >99.9% nucleotide identities. Conversely, Illumina data assembled using three different assemblers resulted in fragmented assemblies, sharing only 92.2-100% and 92.0-100% nucleotide identities to chromosomes I and II reference sequences, respectively, with no indication that the *B. pseudomallei* genome consisted of two chromosomes with four copies of ribosomal operons. Among all assemblies, the PacBio assembly recovered the highest number of core and virulence proteins, housekeeping genes based on whole-genome multilocus sequence typing (wgMLST). Most notably, assembly solely based on PacBio outperformed even hybrid assembly using PacBio and Illumina datasets. PacBio RS II using P6-C4 chemistry is highly robust, cost-effective and should be the platform of choice in sequencing bacterial genomes, particularly for those that are well-known to be difficult-to-sequence.

S17-0001

Gene expression profiling of porcine skeletal muscle satellite cells after challenge with poly I:C

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Porcine satellite cells play a vital role in the construction, development, and self-renewal of skeletal muscle. In this study, porcine satellite cells were exposed to mimic viral infection (poly (I:C)) during proliferation and differentiation phases at 0h, 12h, 24h and 48h time points. The visible changes in phenotypes among untreated and treated porcine satellite cells in the proliferation and differentiation phases were further analysed by RNA sequencing technology. In the proliferation and differentiation phases of porcine satellite cells grown under poly (I:C), 88, 119, 104 and 95 genes were differentially expressed in 0h – 12h treated, 12h – 24h treated, 0h – 24h treated and 24h – 48h untreated comparison libraries, respectively. The GO terms analysis results showed that in the proliferation phase of treated porcine satellite cells, the up-regulated genes related to immune system were highly expressed. Besides, the gene expression associated with muscle structure development, response to growth factor was emerged in the differentiation phase of untreated porcine satellite cells. The biological pathways associated with Influenza A, Toll-like signaling as well as chemokine signaling were revealed through poly (I:C) stimulation of porcine satellite cells. The differentially expressed genes were confirmed by quantitative real-time PCR. Our findings expanded the understanding of gene expression and signaling pathways about the infiltrated mechanism of the virus into porcine skeletal muscle satellite cells.

S17-0002

Induction and activation of cGAS DNA sensor by Sindbis virus

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During viral infection, production of type I interferons is stimulated to trigger local antiviral response. This process is initiated by the recognition of pathogen associated molecular patterns (PAMPs) by host pattern recognition receptors. Cytosolic nucleic acids are PAMPs primarily sensed during viral infection by DNA and RNA sensors. Cyclic GMP-AMP (cGAMP) synthase (cGAS) is generally accepted as a key cytosolic DNA sensor, which synthesizes cGAMP upon DNA binding. cGAMP then binds to stimulator of IFN genes (STING) to elicit type I IFN response. From *in silico* cGAS modeling, it is expected that cytosolic RNA cannot activate cGAS. However, cGAS confers protection against dengue virus, which indicates that cGAS activation is involved during some RNA virus infection. To elucidate cGAS-dependent antiviral activity against *Togaviridae* family, Sindbis virus (SINV) is used as a prototypic model to test the hypothesis that infection of togaviruses activates cGAS. At an early phase of SINV infection, cGAS mRNA and protein expression was found to be induced in an IFN-independent manner. Ectopic expression of cGAS and STING restricted SINV replication in HEK293T cells that are deficient

of both cGAS and STING, while depletion of cGAS impaired type I IFN production and facilitated SINV replication. Both gain-of-function and loss-of-function assays suggested that cGAS is required for type I IFN-dependent restriction of SINV replication. Mechanistically, SINV infection resulted in reactivation of endogenous retroelements, leading to the accumulation of cytosolic DNA reverse transcribed from endogenous retroelement mRNAs but not from RNA genome of SINV. Taken together, this study revealed a role for DNA sensor cGAS in the innate antiviral immune response against SINV and a new mechanism by which SINV activates cGAS by elevating the level of cDNA of endogenous retroelements in the cytoplasm of host cells. Our work provides another example for cGAS activation by an RNA virus.

S17-0003

Investigation of the Immunomodulatory Role of SIRT5 in Rheumatoid Arthritis

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Sirtuin5 (SIRT5) is a mitochondrial NAD-dependent deacetylase, which catalyzes acetylation, succinylation, malonylation, and glutarylation for its substrate proteins. SIRT5 has recently been found to modulate anti-inflammatory pathways via suppression of p65 activation by SIRT2 deacetylation. Additionally, LPS decreases both SIRT5 expression and levels of NAD+ in macrophages, while increasing protein succinylation, suggesting that sesuccinylase activity of SIRT5 was inhibited by LPS. Furthermore, SIRT5 desuccinylation could blocks IL-1β production in lps-activated macrophages by regulating Pyruvate kinase M2 (PKM2). Therefore, pharmacologically activation of SIRT5 with SIRT5-selective activators may provide a new alternative approach for treatment of RA. To address the role of SIRT5 in RA, construction of an adenoviral vector carried the rat gene SIRT5(Ad-SIRT5-EGFP) was injected in articular cavity of rats with different doses. Animal joint swelling, foot scores and other indicators were observed. Animal blood, synovial and immune organs were analyzed at 4 weeks experimental period. Also the change of cytokines, chemkines was detected. After induction of AIA, rats injected with Ad-SIRT5-EGFP could showed potential for anti-inflammation. SIRT5 overexpression inhibited arthritis severity in hind paws based on arthritis score, increased paw volume and radiographic indicators like BMD, TMD and micro-CT score. It significantly suppressed the level of cytokines IL-1β, IL-6, TNF-α, MMP-3, CPM-1 in plasma. Morever, injection of Ad-SIRT5-EGFP inhibited differentiation and bleeding of immune organs like thymus, kidney, spleen and kidney. The in vivo experiment demonstrated that SIRT5 may become one of the therapeutic target for RA treatment.

S17-0004

Mutations of p53 Tumor Suppressor Gene Contribute to MDR in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is affecting both the developed and developing countries causing severe clinical consequences in patients which lead eventually to the damages of joints and other extra-articular tissues. Rheumatoid arthritis synovial fibroblast (RASF) or rheumatoid arthritis fibroblast-like synoviocytes (RAFLS) within the RA synovium is the key regulatory cells of disease progression of RA,

which show the typical insensitivity towards death signaling and drug treatment that observed in many malignant cancers and is capable of spreading to and damaging the surrounding or distal normal cartilage tissues. Of note, TP53 mutations are closely associated with apoptosis- and drug-resistance and invasiveness of cancer cells during the course tumorigenesis. Surprisingly, our bioinformatics analysis indicated that 50% (104 out of 207 cases) of RA cases reported in clinical studies are having TP53 mutations, which is similar to cancer patients. Also, the various RA-associated genetic mutants and hotspots mutations of TP53 in RA patients are resembling to those found in many cancer cases. Based on the fact that TP53 mutations are critical to the development of malignancy, such genetic variations may also underpin the pathomechanisms of RA. In particular, the molecular contributions of mutated TP53 in compromising the apoptotic machinery, triggering the cellular mobility, and inducing drug resistance in cancers further suggested such notion. Our preliminary data demonstrated the participation of TP53 mutations in apoptosis-resistant phenotypes of RAFLS, which is insensitive to the standard methotrexate (MTX) treatment, as well as the other anti-arthritic agents such as chlororquine and cyclosporin A. However, studies focusing on the interaction of TP53 mutations and RA pathogenesis are rare. We proposed here a large scale investigation to elucidate the pathological detail of TP53 mutations in the development of RA and potential drug resistance.

S17-0005

The Promising Biomarker of 14-3-3η in Rheumatoid Arthritis

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Rheumatoid arthritis (RA) is one of the most common chronic inflammatory joint diseases. 14-3-3η protein belongs to the family of 14-3-3 proteins that consists of 7 isoforms（α/β, γ, η, δ/ζ, σ and τ/θ). It interacts with more than 200 intracellular proteins, with functions of immune inflammation, stress response, tumor occurrence-among others. Of note,14-3-3η protein can mediate the occurrence of the immune inflammatory response and aggravate the inflammatory response by combining with protein kinase C to regulate the release of inflammatory cytokines. Alternatively, some studies had found that there was a significant relationship between 14-3-3η protein level and MMP in RA, which can promote the joint inflammation and injury process. More recently, it has been demonstrated that the serum 14-3-3η protein was present at significantly common higher levels in patients with erosive RA. As noted above,14-3-3η protein could activate various pro-inflammatory mediators and played an important role on the pathophysiological process associated with RA. There are a few researches reported that 14-3-3η could be a major candidate in RA. In a study conducted on 619 subjects, 14-3-3η protein sensitivity and specificity for RA was 77% and 93% respectively. In the early stages of the disease, the determination of protein 14-3-3η along with rheumatoid factors (RF) and anticyclic citrullinated peptide (anti-CCP) increases the diagnostic rate from 72% (RF + anti-CCP) to 78% (RF + anti-CCP + 14-3-3η). Maksymowych et al. found that the positive rate of 14-3-3η protein in early RA group, and established RA group were 60–82 and 78%, respectively. Meanwhile, the positive rate of RF in early RA groups was 32%, the anti-CCP antibody was 44%. Taken together, it is shown that 14-3-3η protein might be used as a novel and valuable indicator for diagnosis of RA, which might be more valuable than RF and anti-CCP.

S17-0006

V Protein of PPRV Play Vital Roals in Escape from Host Immunity by Inhibiting the Interferons

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Peste des Petits Ruminants Virus (PPRV) causes a highly pathogenic and mortal disease in small ruminants including both domestic and world, such as goats and sheep. The non-structural protein V plays vital roles in escaping from the host immunity by inhibiting the Interferons. In present study, recombinant plasmids were firstly constructed to express the non-structural V gene. Subsequently, real-time PCR is used to investigate the PPRV V protein inhibits the Interferons. The results showed that the expression of IFN-β, and its downstream interferon stimulated genes, such as 56 (ISG56) ,ISG15 and C-X-C motif chemokine（CXCL-10）were down-regulated at the transcriptional levels. Furthermore, 293T cells were pretreated with IFN-α(2b) or supernatants from co-transfected cells, then infected with Sendai virus (SeV), vesicular stomatitis virus (VSV), Encephalomyocarditis virus (EMCV), or Hepatitis E virus (HEV). The results of qPCR suggest that V protein can inhibit the interferons in both endogenous and exogenous levels. In addition, the level of phosphorylated IFN regulatory factor 3 (IRF3) stimulated by virus was enhanced in the absence of V protein. Collectively, we have already demonstrated that PPRV V protein can inhibit the type I interferon pathway. Our studies give a new sight for the future studies to understand how the PPRV escape from host immunity.

S18-0001

Evaluation of the sub-acute toxicity of Licorice - Sargassum extract in Sprague-Dawley rats: biochemical, histopathological, and pharmacokinetic studies

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The aim of this study was to investigate the toxic mechanism of compatibility application of sargassum and licorice by sub-acute toxicity test and pharmacokinetic studies. Rats were orally administrated by sargassum, licorice or licorice-sargassum extract for 4 weeks, respectively. Drug toxicity was assessed according to serum biochemical indicators and histological examination. Following 4-weeks treatment, serum biochemical indicators (ALT, AST, ALP, CK, HBDH, LDH, TG, GLU, BUN) of the licorice-sargassum extract group (SL) were higher than those of the control group ($P<0.01\sim0.05$). Histological examination showed that inflammatory responses were observed in hearts, livers and kidneys in the SL group rats. In order to investigate the possible toxic mechanism of licorice-sargassum extract, pharmacokinetic studies of six components of licorice in rats were performed. A rapid and reliable UPLC-TQ/MS method was developed and validated to simultaneously determine the following six main components of licorice in rat plasma: liquirtin, isoliquiritin, liquiritigenin, isoliquiritigenin, glycyrrhizic acid (GL), and glycyrrhetinic acid (GA). Compared with the licorice group, the combination of sargassum and licorice significantly increased the plasma concentration of GA, evidenced by increased Cmax and AUC ($P<0.01\sim0.05$). The toxic effects of licorice-sargassum were presumed to result from the increased body burden of GA influenced by sargassum.

S18-0002

Statins induce lactate accumulation resulting in the adverse effect with muscle symptoms

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Statins are the most widely using clinical prescribed lipid-lowering drugs currently. However, 7% to 29% of patients receiving statin therapy reported to appear statin-associated muscle symptoms (SAMS). As the well-known major disturbing adverse effects of statins, the mechanism of SAMS is still not fully understood. Notably, previous animal studies and clinical reports have asserted that high lactate level was association with SAMS. Here, we sought to investigate how increasing lactate production contributed to SAMS. Moreover, we hypothesized that lactate can be a potential therapeutic target for SAMS, and look for possible therapy strategy. In this study, C57BL/6J mice were pre-treatment lovastatin for 30 days and tested the swimming endurance, serving as a behavioral readout of SAMS. Measured mice's blood and muscle lactate levels at indicated times. In order to confirm the in vivo findings and clarify how statin regulated lactate production in molecular pathway, a series of metabolic and molecular assays were performed on skeletal muscle cell lines A-204 and C2C12. At first, we demonstrated the raised blood and skeletal muscle lactate level was related to the reduction of exercise capacity in lovastatin treatment mice. Next, we verify statin can drive glycolysis to force lactate production elevation in skeletal muscle cells and simultaneously discovered that stains reduced basal p53 proteins level. Mechanically, we characterize p53 was a transcriptional regulation factor of muscle

specific glycolytic enzyme β-enolase. At last, we used dichloroacetate (DCA) co-administered with lovastatin to mice and found that their exercise capacity and lactate levels were back to normal. We conclusions that statins can via p53/β-enolase axis to elevated lactate production, which contributes to the occurrence of muscle symptoms. (This work was supported by grant from the Science and Technology Development Fund of Macau, project code: 034/2015/A1)

S19-0001

Withania somnifera targets Interleukin-8 and Cyclooxygenase-2 in human prostate cancer progression

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Prostate cancer (PC) is a common non-cutaneous malignancy in men. The incidence of PC is increasing at an alarming rate across the globe. Progression of PC is associated with elevated levels of Interleukin-8 (IL-8) and Cyclooxygenase-2 (COX-2) in malignant cells. Also their elevated circulating levels promote the disease progression from androgen dependent to independent state. Thus inhibiting the expression of IL-8 and COX-2 would be a promising target in the development of prostate cancer therapeutics. The objective of the current study was to investigate the inhibitory effects of *Withania somnifera (WS)* extract on androgen independent prostate cancer cell line (PC3) and to compare the expression of IL-8 and COX-2 in prostate biopsy tissues. We observed a significant decrease in the cell viability with an IC50 of 10µg/ml. Expression levels of IL-8 and COX-2 in prostate biopsy tissue samples and in PC3 cells were predominantly high; however the lowest dose of *WS*significantly inhibits the enhanced expression of IL-8 and COX-2 in PC3 cells in 24hrs. Further *WS* extract (10µg/ml) significantly arrest the cell cycle progression in G2/M phase, which was evident from the rapid accumulation of PC3 cells in this phase. Our results indicate the inherent metastatic and selective inhibitory potential of *WS* against prostate cancer. *Withania somnifera* may be a good therapeutic agent in addition to the existing drugs for PC. Further studies with more prostate tissues are warranted.

S19-0002

Effect of FungalBacube supplementation on weight loss in overweight and obese people

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Gut microbiota and some metabolites are very important for maintaining body weight. Modulation of gut microbiota by probiotics combined with prebiotics may result in weight loss and thus help in obesity treatment. The effect of FungalBacube (FBC) supplementation on weight loss in overweight and obese people was investigated. In a double-blind, placebo-controlled, randomized trial, each subject consumed two capsules per d of either a placebo or a FBC formulation including 3 probiotics with yeast hydrolysate, stachyose tetrahydrate and L-carnitine as prebiotics per capsule. Body weight and body fat mass were measured at baseline, at week 8 and at week 16. By the eighth week, the reductions in energy intake in the FBC group were significantly greater than those in the control group ($P < 0.005$). The body weight and body mass index (BMI) were significantly reduced by week 16 compared with baseline in the FBC group, and these differences were significantly greater than those in the control group for both body weight and BMI ($P < 0.001$). The results show that FungalBacube supplementation can help overweight and obese people to achieve sustainable weight loss. The corrected body mass index (cBMI) and body fit index (BFI) were also proposed to classify overweight and obesity in adults. cBMI is defined as BMI divided by the constant 20. For Chinese adults, overweight and obesity can be defined as follows: overweight is a cBMI greater than or equal to 1.2; and obesity is a cBMI greater than or equal to 1.5. BFI

is defined as the square root of a person's weight in kilograms divided by his height in meters, and the result divided by constants 6 or 5.7 for men and women, respectively. The BFI for standard body weight (SBW) adults varies slightly with ages 21 to 66 y (0.96~1.05).

Body fit index,FungalBacube,Obesity,Prebiotic,Probiotic

S19-0003

Metabolite Profiles of Caudatin-2,6-dideoxy-3-O-methyl-β-d-cymaropyranoside in Zebrafish by Ultraperformance Liquid Chromatography Quadrupole Time-of-flight Mass Spectrometry

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Caudatin-2,6-dideoxy-3-*O*-methyl-*β*-d-cymaropyranoside (CDMC), a C-21 steroidal glycoside isolated from the root of *Cynanchum auriculatum* Royle ex Wight, has been shown to possess antitumor activities in our previous studies. However, the routes and metabolites of CDMC *in vivo* remained largely unknown. In this study, the zebrafish model organism was firstly used to clarify the metabolism of CDMC *in vivo*. The ultraperformance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/Q-TOF-MS) technique combined with MetabolynxTM software was applied to identify the metabolites of CDMC in zebrafish after exposure for 24 h. Ten metabolites of CDMC were found or tentatively identified based on MS and MS/MS data. The results suggested that hydroxylation, hydrogenation, dehydrogenation, demethylation, dehydroxylation and deglycosylation were the main metabolic processes of CDMC in zebrafish. Our study certified that the zebrafish model can wonderfully imitate the current models in explaining metabolic pathways of steroid compounds with the advantages of lower cost, far less amount of compounds needed, easy operation and high performance. Furthermore, zebrafish model can reveal comprehensive metabolic routes *in vivo* and can quickly predict the metabolism of Chinese herb components, especially those of trace compounds.

S19-0004

Total C-21 steroidal glycosides, isolated from the root tuber of Cynanchum auriculatum Royle ex Wight attenuates hydrogen peroxide-induced oxidative injury and inflammation in L02 cells

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Total C-21 steroidal glycosides (TCSGs), isolated from the root tuber of *Cynanchum auriculatum* Royle ex Wight, have been reported to exert many effects including liver protection and antioxidant properties. In order to investigate the potential mechanism underlying the protective liver function of TCSGs, the present study used the human normal liver cell line L02 to evaluate the effect of TCSGs on H2O2-induced oxidative injury and inflammatory responses. L02 cells were pre-treated with different concentrations of TCSGs, followed by exposure to 1.5 mM H2O2. Cell viability was determined. Levels of ALT, AST, LDH and NO were measured. The activities of SOD, CAT, GSH-Px and the production of MDAwere also determined. Intracellular reactive oxygen species (ROS) levels were detected using a fluorescent probe. H2O2-induced oxidative toxicity was attenuated following TCSGs treatment, as indicated by the increased cell viability, decreased levels of ALT, AST, LDH, NO, MDA and ROS, and the promoted activities of SOD, CAT and GSH-Px. To further explore the possible mechanism of TCSGs,

the nuclear factor erythroid 2-related factor 2 (Nrf2) and NF-kB pathways were examined. The results revealed that TCSGs treatment markedly induced Nrf2 nuclear translocation and upregulated the expression of HO-1 in L02 cells. In addition, pre-treatment with TCSGs inhibited the NF-kB signaling pathway by blocking the degradation of the inhibitor of nuclear factor kBα (IkBα), as well as reducing the expression of TNF-α, IL-6, iNOS and COX-2. These results demonstrated that TCSGs could protect L02 cells against H2O2-induced oxidative toxicity and inflammatory injury by increasing the expression of Nrf2 and HO-1, mediated by the NF-kB signaling pathway.

S19-0005

Modulation of cell metabolism and redox capacity as new anti-cancer strategy

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Increasing evidences suggesting that dysregulation of metabolism and redox capacity is implicated to cancer pathogenesis and progression. Complex crosstalk between the oncogenic pathway and metabolic pathway making the current mono-targeting target-based therapies unsuccessful due to ultimate development of drug resistance. Using Chinese herbal medicine (CHM) to holistic modulate cancer metabolism and redox balance is emerging as a promising anti-cancer treatment strategy. Non-small cell lung cancer has a poor survival rate due to frequently observed drug resistance. Epidemiological studies showed that Diabetes patients under Metformin prescription is less likely to develop lung cancer, indicating that glucose and lipid metabolism is linked to lung cancer. Previously studies from our group has identified SREBP1 which are overexpressed in lung tumors, contributing to lung cancer growth. We found that a CHM, berberine, can inhibit lung tumor growth via suppression of lipogenesis and activation of AMPK/mTOR pathways, via induction of autophagy and cell cycle arrest. In addition, Chinese herbal medicine can modulate the Redox microenvironment, resulting in effective cancer inhibition. Sanguinarine, our newly proven NADPH oxidase 2 (NOX2) activator, significantly elevates reactive oxygen species (ROS) levels in gefitinib resistance NSCLC, leading to selectively induction of EGFR mutant protein over-oxidation and degradation. In additional to using an oxidase activator, an alternative approach using Shikonin, a TrxR reductase inhibitor, also leading to accumulation of ROS, selectively killing gefitinib resistance NSCLC. Taken together, with multiple supporting examples, modulating cancer metabolism and redox balance is a new therapeutic strategy for treating NSCLC. In the future, combinational of these Chinese herbal medicines or together with western mono-targeting drug is a promising drug development strategy.

S19-0006

Krukovine suppresses KRAS-mutated lung cancer cell growth and proliferation by inhibiting the RAF-ERK pathway and inactivating AKT pathway

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Oncogenic activation of the KRAS gene via point mutations occurs in 20% to 30% of patients with non-small cell lung cancer (NSCLC). The RAS-RAF-ERK and RAS-PI3K-AKT pathways are the major hyper-activated downstream pathways in RAS mutation, which promotes the unlimited lifecycle of cancer cells and their metastasis in humans. However, the success of targeted therapy is restricted by many factors. Herein, we show a new pharmacological KRAS signaling inhibitor krukovine, which is a small molecular bisbenzylisoquinoline alkaloid isolated from the bark of *Abutagrandifolia* (Mart.) Sandw. (Menispermaceae). This alkaloid targets the KRAS downstream signaling pathways in different NSCLC cell lines, such as H460 and A549, which are established by KRAS mutations. In the present study, we initially investigated the anti-cancer activities of krukovine in KRAS-mutated NSCLC cell lines, as well as KRAS wild type cancer cell line and normal lung cell. Results indicated that krukovine can inhibit the growth and dose-dependently inhibit the colony formation capacity and wound healing ability of H460 and A549. This cytotoxic effect is associated with the induction of cell apoptosis and G1 arrest in those cell lines. Krukovine treatment also suppressed the C-RAF, ERK, AKT, PI3K, p70s6k and mTOR phosphorylation in H460 and A549. This finding suggests that krukovine represses the growth and proliferation of KRAS-mutated cells by inactivating AKT signaling pathway and down-regulating the RAF-ERK signaling pathway. This study provides detailed insights into the novel cytotoxic mechanism of an anti-cancer compound from an herbal plant and promotes the anti-cancer potential of krukovine in NSCLC with KRAS mutation. (Acknowledgements: This work was supported by Macao Science and Technology Development Fund Project No: 082/2013/A3, 082/2015/A3, 0003/2018/A1, 130/2017/A3 and 046/2016/A2).

S19-0007

Treatment with PDEδ inhibitor NHTD suppresses tumor growth and enhances immune function in KRAS mutant lung cancer by modulation of gut microbiota

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Interfering with the binding of the PDEδ to KRAS provides a novel opportunity to inhibit tumor growth in KRAS mutant lung cancer. In our previous study, we reported a novel PDEδ inhibitor, (E)-N'-((3-(tert-butyl)-2-hydroxy-6,7,8,9-tetrahydrodibenzo[b,d]furan-1-yl)methylene)-2,4-dihydroxybenzohydrazide(NHTD), impairing KRAS-PDEδ interaction, thereby affecting the progression of KRAS-mutant lung cancer. Here we reported that treatment of KRAS transgene lung cancer mice with NHTD prevented tumor growth. Morerover, NHTD-treated mice showed increased the "favorable" gut microbiota (eg. high diversity and relative abundance of *Akkermansia*, *Butyrivibrio* and *Faecalibacterium*). Immune profiling showed a significant increase of CD8+ T cells in spleen and tumor in NHTD-treated mice. Together, our results indicated NHTD may modulate the gut microbiota and improve cytotoxic T cell function against KRAS mutant lung cancer.

S19-0008

Study on the inhibitory effect of Sanguisorba officinalis extract on the Wnt/β-Catenin signaling pathway in colorectal cancer cells

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In recent years, the interest in Sanguisorba officinalis (named "DiYu" in Chinese, DY) has been sparked in its anti-cancer activity. In an effort to understand molecular events contributing to the anti-proliferative activity of DY, we investigated its inhibitory effect against Wnt pathway in HT29 human colorectal cancer cells. Moreover, tannins (RZ) and saponin (ZG) fractions were identified as the potential active components and have been studied as well. Western blot results suggested that DY and RZ significantly down-regulated the amount of cytoplasmic β-catenin protein. Furthermore, DY effectively inhibited two targeted gene levels of Wnt pathway, namely, c-Myc and CyclinD1. And in terms of ZG, there's no obvious inhibitory effects presented. On the contrary, it even significantly up-regulated CyclinD1 expression. As CyclinD1 plays an important role in promoting cell proliferation, it may be the potential factor of greatly limiting the Wnt-inhibiting activity. Transcriptomic profiling results demonstrated that DY and RZ only significantly regulated a few of differential expression genes (DEGs), while no DEGs were observed in the ZG group. For the Wnt-related DEGs, DY up-regulated BCL3 and down-regulated PRICKLE4, ANKRD12 and ASPM, while RZ up-regulated ARL4C, EGR1, PPARD, VLDLR, LDLR and downregulated ANKRD12 and ASPM. It suggested the inhibitory activities of DY and RZ towards the Wnt/β-catenin pathway might be relevant to their down-regulation of the described genes. Consequently, our results revealed that the inhibitory effects of DY on the Wnt/β-catenin signaling pathway could contribute to its anti-proliferative effect, which exerted the potency to be developed as promising therapeutic agents to human colorectal cancer. (Acknowledgements: This research was supported by the Macao Science and Technology Development Fund Project No.: 006/2015/A1).

S19-0009

Glycine tabacina ethanol extract ameliorates collagen-induced arthritis via anti-inflammation and anti-oxidation

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Rheumatoid arthritis (RA), an inflammatory autoimmune disorder, is characterized by synovial chronic inflammation, autoantibody production, cartilage and bone destruction. Glycine tabacina (Labill.) Benth has been used as a traditional herbal medicine to treat RA and joint infection. However, very few studies have been carried out to reveal pharmacological activities of this species. Therefore, this study aimed to investigate the anti-arthritic effect of ethanol extract of G. tabacina (GTE) in collagen-induced arthritis (CIA) animal models for the first time, and clarify the underlying mechanism(s). The results showed that oral administration of GTE (1.11, 2.22 and 4.44 g dried weight of herb per kg body weight) significantly ameliorated the arthritic symptoms in CIA rat model, as indicated by the reduction in paws swelling and arthritis index. X-radiographic analysis and histopathological examinations demonstrated that GTE effectively protected the bone and cartilage of joints from erosion, lesion and deformation. The overproduction of IL-1β, IL-6 and TNF-α was remarkably inhibited in the serum of all GTE treatment groups. The restoration of serum T-SOD activity and malonaldehyde levels proved that GTE administration alleviated the oxidative stress in CIA rats. Besides, GTE treatment significantly decreased swelling volume and thickness of arthritic paw as well as serum levels of TNF-α and IL-1β in CIA mice model, further confirming its inhibitory effect on RA progression. In LPS-induced RAW264.7

macrophages, GTE treatment significantly inhibited the expression of iNOS, production of inflammatory mediators (nitric oxide, IL-6, TNF- α) and nuclear translocation of NF-κB p65. Taken together, GTE is a potential effective candidate for the prevention and treatment of RA, which executes anti-RA effect potentially through inhibiting oxidation and NF-κB signal pathway activation and subsequent inflammation.

S19-0010

Anti-proliferative activities of the pro-oxidant T001 on breast cancer cells

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T001 is a newly purified compound from marine fungus *Chondrostereum sp* in *Sarcophyton tortuosum* from the South China Sea. The compound has been reported to have anti-proliferative activities, but the underlying mechanisms remain elusive. Here, we provided a systaltic evaluation of the anticancer activities and insights into the mechanisms of action of T001. The compound showed potent growth inhibitory activities against different cancer cell lines with IC50 ranging from 2.00 uM to 25.12 uM. The anti-proliferative activity of T001 was further confirmed with colony formation assay. Mechanismly, T001 induced apoptosis and increased the Sub-G0/G1 fractions of MDA-MB-231 cells as analyzed by flow cytometry and verified by western blotting analysis of apoptosis marker cleaved nuclear poly (ADP-ribose) polymerase (cPARP). Meanwhile, T001 prevented the xenograft growth of MDA-MB-231 cells in nude mice. Furthermore, T001 induced the Nrf2 luciferases by the cancer pathway analysis of NanoLuc and the Nrf2, γ-H2AX, Ho-1 protein expression by western blotting. The anti-proliferative effect of T001was enhanced when combined with Tin Protoporphyrin IX dichloride, an inhibitor of HO-1. Furthermore, we found that T001 blocked the oxygen consumption rate (OCR) and increased the reactive oxygen species (ROS) level of MDA-MB-231 cells by seahorse and DCF staining, respectively. In summary, T001 inhibited the mitochondrial function, resulting in increased oxidative stress, which ultimately leads to apoptosis. Our study shed light on new a mechanism for the anti-breast cancer activities of T001 and feasibilities to develop a new anti-breast cancer agent. (This work was supported by grant from the Science and Technology Development Fund of Macau, project code: 034/2015/A1)

S19-0011

Radix Polygalae: a medicinal herb modulating the aging gene expression in aged mouse model

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Radix Polygalae (RP) is a common Chinese herbal medicinal plant prescribed for treatment of forgetfulness, anxiety, insomnia and depression in the Chinese herbal prescription. Our published data have revealed the neuroprotective role of RP in the clearance of mutant huntingtin and α-synuclein, which is responsible for the pathogenesis of Huntington's Disease and Parkinson's Disease respectively. As

neurodegenerative disorders are highly related to the immunal-regulatory and aging process, in the current study, we have further extended to study the immunal-regulated anti-aging properties of RP. To this end, we have performed a PCR-array to investigate the immunal-regulated anti-aging effect of RP on 96 selected genes regulating the genomic instability, telomere attrition, mitochondrial dysfunction, proteostasis, laminopathies, neurodegeneration & synaptic transmission, epigenetic alterations, nucleic acid binding, inflammatory response, cellular senescence and oxidative stress. The aged mice were provided with daily drinking water with or without RP at the age of 30 weeks old for 80 consecutive weeks. Samples were then harvested for RNA extraction, reverse transcription and PCR array analysis. The gene profiles in aged mice were compared to normal young mice in both RP treated and untreated group. Results: 16 genes responsible for triggering inflammatory and oxidative stress response were upregulated in aged models. Among them, 14 genes were resumed to basal young level after long term RP treatment. Our result has provided a detailed gene profile on the changes of a panel of aged and immunal-regulatory related genes, upon treating with the traditional neuroprotective herbal medicine RP, which suggests the possible mechanistic action of RP in traditional herbal prescription by modern pharmacological study.

S19-0012

Identification of novel RORγt antagonists from natural product

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Accumulated evidence showed that autoreactive T cells contribute to the pathology of various autoimmune diseases such as multiple sclerosis (MS) and rheumatoid arthritis (RA) which are amongst the most common autoimmune diseases in the northern hemisphere. According to literature reports, Th17 mainly related to progression of autoimmune disease. Therefore, inhibition of IL-17 production is considered to be an important strategy for the treatment of autoimmune diseases. It is well known that the retinoic acid-related orphan nuclear receptor γt (RORγt)/RORγ2 is a major regulator of the development of helper T (Th17) cells that produce interleukin-17 (IL-17). In the current study, we investigated the binding affinity of SKL1, SKL2 and SKL3 on RORγt due to its crucial role in Th17 differentiation. The results demonstrated that SKL1 and SKL3 could directly bind to RORγt except SKL2, although these compounds share the similar chemical structure. To further determined whether SKL1 and SKL3 could inhibit Th17 differentiation *via* binding to RORγt, CD4+ T cells were sorted from splenocytes and polarized to Th17. The results showed that SKL1 and SKL3 could inhibit Th17 polarization, indicating that both of them suppressed Th17 differentiation as RORgt reverse agonists. These findings indicate that SKL1 and SKL3 have the potential to be developed as RORγt-targeted therapeutics for the treatment of autoimmune diseases. (Acknowledgments: This work is supported by Macau Science and Technology Development Fund 0017/2018/A1)

S21-0001

Syllable convergence during ontogeny in Hipposideros larvatus

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During ontogeny, Infants make rapid progress from their earliest, immature vocalizations to mature speech. Whether the changes of acoustic call structure were purely a consequence of physical maturation whether environmental factors or vocal production learning has been debated for decades. Bats are altricial, gregarious, and highly vocal animals. The complex vocal communication requires that auditory systems and neurobiology are exquisitely tuned to acoustic information. As such, they are important animal models for bioacoustic analyses of language-relevant traits. We report the phenomenon of syllable convergence in *Hipposideros larvatus* by analyzing changes to their isolation calls during ontogeny. Our findings indicate that composites in adult social calls originate from isolation calls. As pups develop, the interval between simple syllables gradually shortens and different types of simple syllables combine into composites for social communication. To our knowledge, this is the first study to report this phenomenon in CF-FM bats, and it might be a special feature of isolation call development in *H. larvatus*.

S21-0002

Prenatal development supports a single origin of laryngeal echolocation in bats

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Bat laryngeal echolocation is considered as one of the most complex and diverse modes of auditory sensory perception in animals and its evolutionary history has been the cause of many scientific controversies in the past two decades. To date, the majority of scientific evidence supports that bats (Chiroptera) are divided into two subordinal groups: Yinpterochiroptera, containing the laryngeal echolocating superfamily Rhinolophidae as sister taxa to the non-laryngeal echolocating family Pteropodidae; and Yangochiroptera, containing all other laryngeal echolocating lineages. This topology has led to an unanswered question in mammalian biology: was laryngeal echolocation lost in the ancestral pteropodids or gained convergently in the echolocating bat lineages? To date, there is insufficient and conflicting evidence from fossil, genomic, morphological and phylogenomic data to resolve this question. We detail an ontogenetic study of fetal cochlear development from seven species of bats and five outgroup mammals and show that in early fetal development, all bats including the non-laryngeal echolocating pteropodids have a similarly large cochlea typically associated with laryngeal echolocation abilities. The subsequent cochlear growth rate in the pteropodids is the slowest of all mammals and leads to the pteropodids and the non-echolocating lineages eventually sharing a similar cochlear morphospace as adults. The results suggest that pteropodids maintain a vestigial developmental stage indicative of past echolocation capabilities and thus support a single origin of laryngeal echolocation in bats.

S24-0001

Effects of red and blue lights under CO2 elevation on some sweet pepper traits

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Light and CO2 are the main components of photosynthesis. To investigate the impact of light quality on pepper (*Capsicum annuum* L.) growth and development we performed an experiment with supplementary light-emitting diode (LED) of red (R, peak wavelength 660 nm), blue (B, peak wavelength 470 nm) and the combinations of these lights (50% red and 50% blue) under CO2 enrichment (1000 ± 50 mol mol−1). The results revealed that light quality significantly improved morphological and physiological traits of pepper, reduced the period of sowing seeds to harvest and increased yield. The highest plants were found under combinational lights (LED- R+B) with average height of 210 cm. The maximum stem thickness was found under blue light (10.48 mm). Leaf area increased significantly under monochromatic red light (6.11 cm2). Fresh and dry weight of leaf, stem and fruit were the highest under blue color. Red light considerably increased photosynthetic pigments such as Chlorophyll a, *b*, total and carotenoids. Under LED- R+B pepper plants produced the highest fruits (29), ascorbic acid (91 mg/100 ml) and PAL enzyme activity. Considering the highest fresh and dry weight of leaf and fruit and the mean number of pepper fruits under blue light, it is suggested that blue color is the most beneficial for pepper cultivation in a growth chamber. Furthermore, using DIAlux software (DIAL GmbH, Germany) we calculated that 24 blue color lumps is needed for 10 * 10 m2 room. It is concluded that artificial light in particular monochromic blue LED can dramatically improve the pepper characters and financial returns for farmers.

S24-0002

Effects of ozone stress on N and P stoichiometry of soybean

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Open-top chambers (OTCs) were utilized to understand the effect of ozone stress on N and P the stoichiometric characteristics in different organs of soybean (Glycine max.) under different O3 concentration at (80±10 nmol·mol-1 and 110±10 nmol·mol-1). The results showed that
compared with the control treatment, with the increase of ozone concentration and extension of growth period, N content declined in roots and stems, while it firstly increased and then decreased in leaves in the ozone stress treatments; P content increased in roots at branching and podding, and it declined at flowering, while it increased in stems, and firstly declined and then increased in leaves in the ozone stress treatments; N/P firstly elevated and then declined in leaves and roots, and decreased in stems in ozone stress treatments. There were no correlations among roots, stems, and leaves in N content ($P<0.05$), while there were significant correlations among roots and stems in P content ($P<0.05$). N content was significantly correlated with P content in roots and stems ($P<0.05$); N content and N/P of different organs followed the order: leaves> roots > stems，but there was no significant difference in P content among

different organs．Meanwhile，the coefficients of variation for N content followed the order: stems> roots>leaves, and the coefficients of variation for N/P followed the order: roots>leaves >stems, but the coefficient of variation for P content was similar in different organs. These results indicated that with the increase of ozone stress, soybean growth is mainly limited by N and P stoichiometry of soybean.

S24-0003

Overexpression of OsARD1 improves submergence tolerance by the increase of ethylene in rice

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Acireductone dioxygenase (ARD) is a metal binding metalloenzyme and involved in the methionine salvage pathway. In rice, *OsARD1* binds Fe2+ and catalyzes the formation of 2-Keto-4-methylthiobutyrate (KMTB) to produce methionine, which is an initial substrate in ethylene synthesis pathway. Here, we report that overexpression of *OsARD1* enhances the tolerance to submergence stress in rice. *OsARD1* is strongly induced by submergence stress stresses. Moreover, *OsARD1* exhibits high expression in senescent leaves. Further experiments show that water holding capacity is enhanced and the stomata and trichomes on leaves increase in transgenic plants overexpressing *OsARD1*. The experiments of submergence stress and the growth of etiolated seedlings in the dark display increased shoot elongation and inhibition of root elongation in transgenic plants overexpressing *OsARD1*, similar to the phenotype of increasing ethylene in rice. The measure of ethylene showed that the level of ethylene is significantly increased in transgenic plants overexpressing *OsARD1*. Further experiments show that the ethylene synthesis related genes are upregulated in transgenic plants overexpressing *OsARD1*. Taken together, the results provide the understanding that *OsARD1* plays important roles in submergence tolerance.

S25-0001

Nutrition outweigh defense: Why Myzus persicae (green peach aphid) prefers and performs better on young leaves of cabbage

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Plant leaves of different ages differ in nutrients and toxic metabolites and thus exhibit various resistance levels against insect herbivores. However, little is known about the influence of leaf ontogeny on plant resistance to phloem-feeding insects. In this study, we found that the green peach aphid, *Myzus persicae*, preferred to settle on young cabbage leaves compared with mature or old leaves, although young leaves contained the highest concentration of glucosinolates. Furthermore, aphids feeding on young leaves had higher levels of glucosinolates in their body, but aphids performed better on young leaves in terms of body weight and population growth. Phloem sap of young leaves had higher amino acid:sugar molar ratio than mature leaves, and aphids feeding on young leaves showed two times longer phloem feeding time and five times more honeydew excretion than on other leaves. These results indicate that aphids acquired the highest amount of nutrients and defensive metabolites when feeding on young cabbage leaves that are strong natural plant sinks. Accordingly, we propose that aphids generally prefer to obtain more nutrition rather than avoiding host plant defense, and total amount of nutrition that aphids could obtain is significantly influenced by leaf ontogeny or source-sink status of feeding sites.

S25-0002

OsHLH61-OsbHLH96-OsJAZ3 complex renders susceptible to brown planthopper through regulating the Pathogen-related genes

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In plants, basic helix-loop-helix (bHLH) proteins form the largest transcriptional factor family. Among them, HLH protein is a small group of atypical member that lacks the basic domain, which function through formation of dimers with bHLH proteins. Although bHLH proteins have been proved to play important roles in plant development and physiology, the function of HLH proteins was rarely studied, not to mention in plant biotic resistance. Brown planthopper (BPH) is a kind of rice-specific insect that cause devastating yield lose each year. Formerly, we identified that a HLH encoding gene, *OsHLH61*, could be highly induced by BPH infestation. In this study, we cloned *OsHLH61* and confirmed its BPH-responsive character. Methyl Jasmonic acid (Me-JA), Salicylic acid (SA) and the precursor compound of JA, cis-12-oxo- phytodienoic acid (OPDA), could also induced expression of *OsHLH61*. We knocked down expression of *OsHLH61* gene by RNA interference (RNAi), and the transgenic plants were susceptible to BPH infestation. RNA-seq analysis revealed that pathogen-related (*PR*) genes in the salicylic acid (SA) signaling pathway that mediate plant immunity were obviously down-regulated in *OsHLH61* RNAi plants. Meanwhile, we identified bHLH096 to be possible interacting factor of OsHLH61, and OsbHLH96 might interact with Jasmonate Zim-Domain3 (OsJAZ3). Overexpression

lines of *OsbHLH096* also have the lower expression level of *PR* genes than wild type. Altogether, we isolated a HLH-bHLH-JAZ complex that might mediate BPH resistance.

S26-0001

NMR Studies of Membrane Proteins Reconstituted in Disulfide-containing Detergents (DCDs)

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Detergents are widely used as membrane mimics in structural studies of membrane proteins (MPs). Solution NMR with MPs can benefit from detergents that form small micelles, which ensures high frequency of the rotational Brownian tumbling for obtaining high-quality spectra. We report here applications of a new class of detergents (DCDs), which contain a disulfide bond in the hydrophobic chain. This design of DCDs affords efficient MP solubilization and stabilization in small micelles.
To evaluate the application of DCDs in NMR studies with MPs, the *E. coli* outer membrane protein X (OmpX) has been reconstituted into DCD-7 and Fos10. 2D [15N,1H]-TROSY spectra and TROSY-type triple resonance NMR experiments of OmpX refolded in the two detergents have been recorded, sequence-specific backbone assignments have been accomplished, and these data were compared for the two detergents.
The result is that the new detergent DCD-7 was successfully used to reconstitute the β-barrel membrane protein OmpX. The next step will be to validate applications of DCDs with α-helical membrane proteins, in particular G protein-coupled receptors (GPCRs).

S26-0002

NMR Studies of the Glucagon Receptor System Using Fluorine-19 and Nitroxide Labels

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The 29-amino acid polypeptide hormone glucagon exerts its action of maintaining glucose homeostasis by binding to the glucagon receptor (GCGR), a member of the class B G protein-coupled receptors (GPCRs). Interaction between glucagon and the GCGR is of great physiological and pharmacological importance. Here, using fluorine-19 paramagnetic relaxation enhancement (PRE) nuclear magnetic resonance (NMR), we study the interaction between them. We introduced cysteine residues into discrete sites on a water-soluble glucagon variant, which were then labeled with nitroxide radicals. Using in-membrane chemical modification (IMCM), fluorine probes were attached to cysteine residues introduced onto GCGR TMD (transmembrane domain) constructs. Observations made with these fluorine-19 probes in PRE experiments, reveal information on distances between the paramagnetic moieties to the measured nuclei, and thus provided novel insights on glucagon binding to the GCGR TMD. Our data showed that, even in the absence of GCGR's extracellular domain, glucagon can specifically bind to the GCGR TMD at its extracellular surface. In addition, we also found that there is observable interaction between GCGR TMD with other types of polypeptide hormones. This study demonstrates that fluorine-19 PRE is a valuable alternative for the study of the interactions of GPCRs with their ligands.

S26-0003

19F-NMR Studies of the Drug Aprepitant Bound to Neurokinin-1 Receptors (NK1Rs)

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The neurokinin-1 receptor (NK1R) is a member of the class A family of G-protein coupled receptors (GPCRs). It is found in the nervous system and considered to be of interest as a drug target. Aprepitant is a drug which binds to NK1R, where it acts as an antagonist of NK1R to reduce vomiting during chemotherapy and after surgery. We expressed a NK1R construct of residues 2-335 in sf9 insect cells for NMR studies. We can observe aprepitant in the complex with NK1R through its two -CF3 groups, using 19F-NMR. In our study, two different dynamic features were observed. First, flipping of the aromatic ring carrying the two -CF3 groups was observed in some variants of NK1R. The low frequency of this ring flipping indicats that this kind mobility in NK1R-aprepitant complexes has a high energy barrier. Second, several substates of aprepitant bound to NK1R were observed; All these motions reflect plasticity of the NK1R-aprepitant complex, yielding new insights into ligand-receptor interactions.

S26-0004

Paramagnetic Relaxation Enhancement NMR Studies of the Glucagon-like Peptide-1 Receptor System

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The glucagon-like peptide-1 receptor (Glp-1R) is a member of the class B family of G protein-coupled receptors (GPCRs). The Glp-1R transmembrane domain (TMD) structure in complexes with allosteric modulators, and the full length Glp-1R structure in complex with a peptide agonist were solved by X-ray crystallography, as was the cryo-EM structure of active Glp-1R in complex with a G protein [1-3]. With the availability of the aforementioned crystal structures, complementary dynamics information will further support structure-activity analyses of this polypeptide hormone-receptor system. Here, we studied the interaction between Glp-1 and the Glp-1R TMD by paramagnetic relaxation enhancement (PRE). First, fluorine-19-probes were introduced near the orthosteric binding site of Glp-1R by conjugation of 2,2,2-trifluoroethanethiol (TET) with cysteine-SH groups and site-directed spin labeling was used to introduce a nitroxide spin label at different position of Glp-1 peptide, respectively. Then, nitroxide spin labeled Glp-1 were used to study the interactions with Glp-1R TMD by fluorine-19-NMR experiments. NMR studies suggest that fluorine-19 signals near the orthosteric binding site were attenuated by S-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl) methyl methanesulfonothioate (MTSL) labeled Glp-1, while fluorine-19 signals on the intracellular surface were not affected, which suggests that the Glp-1 peptide can specifically interact with the transmembrane domain of Glp-1R.
In conclusion, the interaction of Glp-1 with Glp-1R TMD was successfully observed with PRE NMR measurements, which provided new sights on the interactions between Class B receptors and their ligands.
Nuclear magnetic resonance,paramagnetic relaxation enhanc,glucagon-like peptide-1 recept

S26-0005

19F NMR of Neurokinin-1 Receptor-Aprepitant Complexes Reveals Conformational Exchange in the Binding Site

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Aprepitant (EMEND), a neurokinin-1 receptor (NK1R) antagonist, was approved by the FDA in 2003 for prevention of chemotherapy-induced nausea and vomiting (CINV). Aprepitant blocks signals by binding to the NK1R in the human brain, which decreases the likelihood of vomiting in patients. Crystal structures of the human NK1R in complex with aprepitant already provided a detailed static picture of how the receptor interacts with the ligand. 19F-NMR of receptor-bound aprepitant shows two peaks corresponding to the two trifluoromethyl groups, which reflects their different microenvironments in the NK1R binding site. The presence of the separate peaks in the 1D 19F-NMR spectra and [19F,19F]-EXSY experiments show that there is conformational exchange in the binding site. Specifically, there are at least two substates of the bound ligand, with exchange frequency in the millisecond range. The bis-trifluoromethyl-phenylethoxy moiety of aprepitant bound to the NK1R receptor does not undergo ring flipping motions at frequencies that are detectable with the experiments used, indicating that *k*ex <10 s-1 or possibly orders of magnitude slower. The binding site environment of the ligand thus imposes a high energy barrier to such mobility. Possible exchange between free and receptor-bound aprepitant is also too slow to be seen by the presently used experiments.

S26-0006

Effects of ammonia-N stress and post-exposure recovery on the histopathology of the gill, liver and kidney of juvenile Myxocyprinus asiaticus

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Excessive ammonia nitrogen (ammonia-N) and eutrophication have been causing a global problem of water pollution, ammonia-N is widely present in the water bodies in aquacultural production, consisting of non-ionic ammonia (or molecular ammonia and free ammonia) and ionic ammonia. For aquatic organisms, ammonia-N toxicity mainly refers to the toxicity from non-ionic ammonia, and ionic ammonia is basically non-toxic. The Chinese sailfin sucker (*Myxocyprinus asiaticus*) exhibits a high death rate caused by factors in the external water environment on its processes of breeding and farming. In this study, we confirmed that non-ionic ammonia is one of the factors and that *M. asiaticus* is poorly tolerant to non-ionic ammonia, we investigated the effects of non-ionic ammonia stress and post-exposure recovery on the gill, liver and kidney tissue structure of juvenile *M. asiaticus*. Compared with other fish species of identical specifications, the juveniles of *M. asiaticus* were poorly tolerant to non-ionic ammonia. Different organs of juvenile *M. asiaticus* respond differently to acute ammonia-N stress, in the following order, from severe to light damages: gill, liver and kidney. After 96 h of post-exposure recovery, all the organs were recovered to varying degrees, indicating that the tissue damages caused under the described conditions were reversible, although 96 h did not allow for a full recovery, for which kidney is the slowest.

S27-0001

Modular Regulation of p53 DNA Binding by iASPP Imparts Target Selectivity

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Interaction of sequence-specific transcription factors (TFs) with cognate DNA sequences is fundamental to development and disease. DNA-binding structural modules of TFs primarily determine their response elements yet many TFs, such as the tumor suppressor p53, regulate subsets of target genes in a context-dependent manner. Hitherto, mechanisms underlying sequence-specific regulation of TFs by direct partner binding remain elusive. Here we identify sequence signatures enriched in p53 response elements regulated by its inhibitor iASPP. Our p53–iASPP crystal structure reveals that iASPP displaces p53 L1 loop, which mediates sequence-specific interactions with the signature-corresponding base, from its DNA-binding position but minimally affects other p53 DNA-recognizing modules. Interaction of oncoprotein E6 from human papillomaviruses coincides with iASPP binding on p53, suggesting a functional, potentially-druggable p53 surface distal from formerly-reported interfaces. Our findings exemplify the mechanisms by which direct interactions of DNA-binding domains with regulatory partners impart DNA-binding refinement and thus target selectivity to TFs.

cancer,DNA binding,p53,target selectivity,iASPP

S27-0002

Proscillaridin A induces DR4 up-regulation and suppresses non-small cell lung cancer tumor growth

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Non-small cell lung cancer (NSCLC) is the predominant histological type of lung cancer and is characterized by the highest mortality and incidence rates among these types of malignancies. Cardiac glycosides, a class of natural products, have been identified as a potential type of chemotherapeutic agent. This study aims to investigate the anti-cancer effects and the mechanisms of action of Proscillaridin A (P.A) in NSCLC cells. *In vitro* sodium-potassium pump (Na+/K+ ATPase) enzyme assays indicated that P.A is a direct Na+/K+ ATPase inhibitor. P.A showed potent cytotoxic effects in NSCLC cells at nanomolar levels. Treatment mechanism studies indicated that P.A elevated Ca2+ levels, activated the AMPK pathway and down–regulated phosphorylation of ACC and mTOR. Subsequently, P.A increased death receptor 4 (DR4) expression and down-regulated NF–κB. Interestingly, P.A selectively suppressed EGFR activation in EGFR mutant cells but not in EGFR wild-type cells. *In vivo*, P.A significantly suppressed tumor growth in nude mice compared to vehicle-treated mice. Compared with the Afatinib treatment group, P.A displayed less pharmaceutical toxicity, as the body weight of mice treated with P.A did not decrease as much as those treated with Afatinib. Consistent changes in protein levels were

obtained from Western blotting analysis of tumors and cell lines. Immunohistochemistry analysis of the tumors from P.A-treated mice showed a significant suppression of EGFR phosphorylation (Tyr 1173) and reduction of the cell proliferation marker Ki67. Taken together, our results suggest that P.A is a promising anti-cancer therapeutic candidate for NSCLC. (Acknowledgements: This work was supported by the Macau Science Technology Development Fund project code: 021/2013/A1, 005/2014/AMJ & 010/2016/A1).

S27-0003

Down-regulating Nrf2 with tangeretin reverses multiple drug resistance cancer: in vivio and in vitro study

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Background: No matter in chemotherapy or molecularly targeted therapy, drug resistance is the main obstacle encountered by current cancer clinical treatment. However, the underlying mechanisms remain to be elucidated. Elevated reactive oxygen species (ROS) has been described to be closely associated drug resistance. Therefore, targeting the abnormal redox signaling may provide a novel anti-cancer strategy to selectively kill resistant cancer cells.

Aims: In this study, we aim to investigate the role of ROS in both chemotherapy and molecular targeted drug resistance cancer and whether it can enhance the efficacy of chemotherapy on drug resistant NSCLC cells. by down-regulating Nrf2 signaling pathway.

Results: Compared with normal cells and non-resistant cancer cells, the ROS and its key regulator Nrf2 were remarkably up-regulated in drug resistant NSCLC cells. Knockdown of Nrf2 to further enhance ROS level substantially increased the apoptotic cells and sensitized cancer cells to anti-cancer drug treatment. Moreover, tangeretin (TG), a flavonoid isolated from traditional Chinese medicine citrus peels, was testified to be an effective Nrf2 inhibitor which can significantly induce Nrf2 degradation and thus suppress Nrf2 signaling pathway, as well as the expression of ATP-binding cassette (ABC) transporter: P-glycoprotein (Pgp). The anti-cancer effect of TG was investigated in both *in vitro* and *in vivo* studies which have demonstrated that suppression on Nrf2 is able to efficiently inhibit the growth of drug resistant cancer cells.

Innovation: We have identified a novel therapeutic strategy to conquer drug resistance in cancer cells via inhibiting Nrf2 and manipulating cellular redox balance.

Conclusion: Targeting Nrf2 to enhance ROS and reduce ABC transporter has promising potential for cancer patients with drug resistance.

S27-0004

Natural product gossypol inhibits non-small cell lung cancer proliferation by targeting EGFRT790M/L858R

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Non-small cell lung cancer (NSCLC) which accounts for approximately 85−90% of lung cancers, has proven difficult to treat due to poorly understood pathological mechanisms. Gain-of-function of primary

mutations of EGFR such as L858R or deletions within exon 19, lead to increased cell proliferation and survival, which increased sensitivity to EGFR tyrosine kinase inhibitors (TKIs). The most common mechanism of acquired resistance is the EGFR gatekeeper T790M mutation in EGFR tyrosine kinase domain, which is observed in approximately 50% of patients with first generation EGFR inhibitor resistance. Despite the fact that many EGFR inhibitors have been developed so far, we can find that most of the irreversible EGFR inhibitors bear either quinazoline or aminopyrimidine core pharmacophores by examining the chemical structures. Consequently, there has urge need to discover EGFR inhibitors with novel scaffold. Here we identified a natural product Gossypol that suppresses the proliferation of NSCLC cell lines and the classical EGFR/AKT/ERK signaling pathway driven by EGFR activated by various oncogenic mechanism. It also dose-dependently induced apoptosis in the EGFR T790M/L858R mutant cell lines. These results, together with the fact that Gossypol have good physical and chemical properties, we believe that this novel EGFR T790M/L858R inhibitor would be a useful pharmacological lead for further development of EGFR inhibitors for NSCLC.

S27-0005

MicroRNA 421 intermediates celastrol-induced apoptosis in lung cancer

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Celastrol, a triterpene extracted from traditional Chinese medicine, was demonstrated to be able to induce apoptosis in several cancer cells. However, its underlying mechanism is unknown. MicroRNAs regulate gene expression through inhibition of translation or degradation of the targeted mRNA. MiR-421 has been found to be significantly up-regulated in cancer cells and is able to promote cell proliferation and migration in cancer. Here, we showed that celastrol significantly inhibited cell viability and colony formation in A549. We have also identified that knockout of β-catenin expression reduced sensitivity of A549 cell to celastrol which inhibited the Wnt/β-catenin signaling pathway. In addition, our study indicated that celastrol promoted the phosphorylation of GSk3β, β-catenin and inhibited the expression of c-Myc and cyclin D1. Finally, celastrol also significantly suppressed miR-421 expression and inhibited miR-421 promoter-driven luciferase activity. Taken together, our data revealed that the effect of celastrol on apoptosis was due to suppress Wnt/β-catenin/miR-421 signaling pathway. (This works was supported by Macau and Technology Development Fund Project No: 082/2013/A3, 082/2015/A3, 0003/2018/A1,130/2017/A3,046/2016/A2,010/2016/A1)

S27-0006

Blocking protective autophagy enhanced Deltarasin-induced apoptosis impaired Kras-PDEδ interaction in lung cancer

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Deltarasin is a recently identified small molecule that can inhibit KRAS-PDEδ interactions by binding to a hydrophobic pocket on PDEδ, resulting in the impairment of cell growth, KRAS activity, and

RAS/RAF signaling in human pancreatic ductal adenocarcinoma cell lines. Since KRAS mutations are the most common oncogene mutations in lung adenocarcinomas, implicated in over 30% of all lung cancer cases, we examined the ability of deltarasin to inhibit KRAS-dependent lung cancer cell growth. Here, for the first time, we document that deltarasinproduces both apoptosis and autophagy in KRAS-dependent lung cancer cells in vitro and inhibits lung tumor growth in vivo. Deltarasininduces apoptosis by inhibiting the interaction of with PDEδ and its downstream signaling pathways, while it induces autophagy through the AMPK-mTOR signaling pathway. Importantly, the autophagy inhibitor, 3-methyl adenine (3-MA) markedly enhances deltarasin-induced apoptosis via elevation of reactive oxygen species (ROS). In contrast, inhibition of ROS by N-acetylcysteine (NAC) significantly attenuated deltarasin-induced cell death. Collectively, these observations suggest that the anti-cancer cell activity of deltarasin can be enhanced by simultaneously blocking "tumor protective" autophagy, but inhibited if combined with an anti-oxidant.

S27-0007

Combined use of PI3k and MEK inhibitors synergistically inhibits lung cancer with EGFR and KRAS mutation

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EGFR and KRAS mutation are the two most common driver mutations in non-small cell lung cancer (NSCLC). Molecular target-based therapy using small molecules like gefitinib has been used for inhibiting EGFR with good initial responses, however drug resistance is common by mono-targeting strategy. KRAS is still an undruggable target currently. As such, developing new drug targeting the downstream of KRAS and EGFR and their crosstalk pathways are ultimately important to treat NSCLC. The present study aims to elucidate the anti-cancer effect of BKM120 and MEK inhibitor (PD1056309) in NSCLC cell lines. By inhibiting EGFR and KRAS downstream P13k pathway using BKM120 significantly inhibit growth of NSCLC cell lines with either EGFR or KRAS mutation. In addition, significant cell cycle arrest and apoptosis induction were observed after BKM120 treatment. Similarly, PC9 and H1650 harbor the same EGFR mutation, however, H1650 is less sensitive to BKM120. Different sensitivity between NSCLC cell lines with same oncogenic mutation suggests that multiple crosstalk pathways exit. Combined usage of BKM120 and PD1056309 synergistically enhanced apoptosis in both A549 and H1650 cells, suggesting crosstalk of MEK pathway with P13k/Akt pathways in both cell lines. Overall, our findings suggest that inhibiting EGFR and KRAS downstream with P13k/Akt inhibitor could be useful for treating NSCLC. However, for NSCLC with crosstalk with other survival pathways, such as MEK pathway, combination treatment is required. (Acknowledgments: This works was supported by Macau and Technology Development Fund Project No: FDCT-16-010-SKL； Project No: FDCT 046/2016/A2)

S28-0001

Signalling pathways (Notch, Wnt, Fgf, Bmp and Shh) involved in hair cell regeneration in the mouse cochlea after gentamicin-induced damage

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Although mammalian hair cells are essentially unable to regenerate after damage, non-mammalian species have a robust capacity for hair cell regeneration. This raises an interesting question: can we induce mammalian hair cell regeneration using the mechanisms by which hair cells are regenerated in non-mammalian cochleae? To identify the precise signalling pathways involved in hair cell regeneration, we analysed the transcriptomic changes in mouse cochleae 3 days after gentamicin damage and compared them with those obtained from chick cochleae at the corresponding time after damage. At this time point, many cells have undergone proliferation, and some new hair cells have been regenerated. The results indicated that 22,086 genes were expressed in the gentamicin- and saline- treated (as control) cochleae of neonatal mice, of which 2,243 had significant differential expression between the gentamicin- and saline-treated cochleae. In addition, some of these differentially expressed genes were grouped into 265 signalling pathways, including the Notch, MAPK (FGF), Wnt, TGF-β (BMP) and sonic hedgehog (Shh), all of which have been shown to play important roles in cochlear embryonic development. However, unlike in the chicken cochlea, most of the pathways in the mouse cochlea were not in the list of the top 50 signalling pathways. To our knowledge, our study provides a relatively complete dataset of candidate genes and signalling pathways involved in hair cell regeneration after damage. By pharmacological inhibition or activation, we found a new co-regulation (a combination of Notch and BMP) that causes hair cell regeneration in the mouse cochleae after damage.

S28-0002

Exploring the Antidepressant Molecular Target and its Application in Depression Treatment via Traditional Chinese Medical Compound Baicalin

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Depression is one of the serious but common psychiatric issues in the current world. Due to lack of understanding for the biological mechanism underlying the development of depression, current treatments usually concentrate on attenuating the pathophysiologies related the disease. Our team identified the new metabolic factor adaptor protein, phosphotyrosine interacting with PH domain and leucine zipper 2 (APPL2), also performs as the negative regulator to adult hippocampal neurogenesis, which considered as the neurobiological hallmark associating with emotional control. We found mice carrying overexpression of APPL2 exhibited the depressive and anxiety-like behaviors as well as the dampened hippocampal neurogenesis. The hyperactivity of glucocorticoid receptor (GR) was shown to have directly resulted from the overexpression of APPL2.

S28-0003

Elucidating the therapeutic potential of human neural stem cells with genetic manipulation of SUFU expression following spinal cord injury

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The majority of spinal cord injury (SCI) cases is due to traumatic injury that leads to irreversible loss of neuronal connections and demyelination of axons, and often results in permanent paralysis. There is currently no treatment for SCI. The limited locomotor recovery is caused by glial scar formation, which consists of reactive astrocytes that form a mechanical barrier to inhibit axonal regeneration. Transplantation of neural stem cells (NSCs) offers a potential strategy for treating SCI, because NSCs have the ability to differentiate into neurons to restore the neuronal circuitry and into oligodendrocytes for remyelination of spared axons. Although previous studies demonstrated the ability of transplanted NSCs derived from pluripotent stem cells to restore locomotion of mice with injured spinal cord, the majority of grafted cells differentiated into a mixed neuronal population including less than 5% of spinal cord motorneurons and the number of oligodendrocytes formed (<1%) was not sufficient for remyelination of spared axons. Here we reveal that targeting the negative regulator of sonic hedgehog (Shh) signaling, Suppressor of Fused (SUFU) expression by shRNA could promote the self-renewal capacity of human NSCs (hNSCs). Upon differentiation, hNSCs with SUFU knockdown (KD) exhibit high propensity to form motorneurons and oligodendrocytes instead of astrocytes. Importantly, grafted SUFU KD hNSCs survive and differentiate into neuronal lineage without forming glial scar and tumors at the lesion site of rodent SCI model. Thus, our findings suggest that targeting SUFU expression in hNSCs could be a viable therapeutic strategy for treating SCI.

S31-0001

Transcriptome sequencing reveals key potential long non-coding RNAs related to duration of fertility trait in the uterovaginal junction (UVJ) of egg-laying hens

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Duration of fertility, (DF) is an important functional trait in poultry production and lncRNAs have emerged as important regulators of various process including fertility. In this study we applied a genome-guided strategy to reconstruct the uterovaginal junction transcriptome of 14 egg-laying birds with long- and short-DF (n=7); and sought to uncover key lncRNAs related to duration of fertility traits by RNA-sequencing technology. Examination of RNA-seq data revealed a total of 9,977 lncRNAs including 2,576 novel lncRNAs. Differential expression (DE) analysis of lncRNA identified 223 lncRNAs differentially expressed between the long- and short-DF groups, with 81 up-regulated and 142 down-regulated ones. DE-lncRNA target genes prediction uncovered over 200 lncRNA target genes and functional enrichment tests predict a potential function of DE-lncRNAs. Gene ontology classification and pathway analysis revealed 8 DE-lncRNAs, with the majority of their target genes enriched in biological functions such as cellular response to cytokine, response to protein homodimerization, reproductive structure development, developmental process involved in reproduction, regulation of protein modification, osteoblast differentiation and ossification, in utero embryonic development, response to cytokine, carbohydrate binding, chromatin organization, response to growth factors, and immune pathways. Differential expression of lncRNAs and target genes were confirmed by qPCR. The discovery of these 2,576 novel lncRNAs in this study significantly expands the utility of the uterovaginal junction transcriptome and our analysis identification of key lncRNAs and their target genes regulating DF will form the baseline for understanding the molecular functions of lncRNAs regulating DF and extend the knowledge of the molecular mechanisms underlying fertility.

Transcript,LncRNAs,Fertility,UVJ,Hens

S31-0002

LFMD: a new method to detect low-frequency mutations without molecular tags

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Since the error rate of next generation sequencing (NGS) is around 1%, it is difficult to identify mutations at and below 1% allele frequencies accurately and efficiently because of low Signal-to-noise ratio (SNR). In order to identify low-frequency mutations (LFMs) and make them more widely used in research and clinical fields, we developed a likelihood-based approach, low-frequency mutation detector (LFMD), which combines advantages of Duplex sequencing (DS) and bottleneck sequencing system (BotSeqS) to maximize utilization of duplicate sequenced reads. Without additional experimental steps, customized adapters and molecular tags, the new method achieves higher sensitivity and specificity with lower cost.

In addition, this method can also be used to improve sensitivity and specificity of other variant calling algorithms by replacing traditional NGS analysis step: removing PCR duplication.

S34-0001

The History of Animal Fecal Transplation Therapy in China

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Chinese ancient medical experts had been recording their clinical experience about fecal transplation therapy, and that is relatively mature. In TCM books, doctors used fecal drugs in practice in general.

Faeces was recorded 4000 years ago in the Oracle Bones Inscriptions in Shang Dynasty, which was the oldest data of Chinese fortunetelling. It had published a series of papers based on the data of more than ten species on human fecal medicine in dosage form. Next, the researcher' animal faeces findings will be released in a paper published. That add up to more than one hundred kinds of faeces. It is classified as birds, beasts, insects and fish and other classes. According to historical records and preliminary statistics, it includes trogopterus dung, feces of silkworm, larvae bat, wild rabbit, male sparrow, male rat, earthworm, white horse, sheep, white dog, antelope, cow, white duck, magpie, male fox, tiger, wolf, deer, wild elephant, goose with grey feather, hytrix hodgsoni, agkistrodon halys, cat, camel, cetacean, tapir, white pigeon, bufonid, macaque, carve, donkey, stork, leopard cat, goat, lion, otter, cormorant, deer fetus, pig and dog fetus, cynthopsis, ostrich, crane, turtledove, pheasant, peacock, stork, silkworm moth etc. There was very strictly procedure in collection and processing, especially some fecal herbs that were processed excluding separation and purification.

It was been guidanced by the theory of TCM, including of donators, processing of faeces, preserving, dosage form, adaptation diseases, therapeutic mechanism, contraindication and side reaction. There were many of medicince delivery route based on different diseases. The faeces that broad and abundant clinical treatment information to the modern medicine has accumulated a large number of enlightenment. It is an extremely valuable wealth in traditional Chinese medicine and even the world medicine.

S34-0002

Investigation of the effects of Pogostemon cablin on gut microbial composition in C57BL/6J mice

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Pogostemon cablin (Blanco) Benth. (PC) is an important herb used in the clinical practice of traditional Chinese medicine (TCM). The pharmacological studies of PC have also revealed several biological activities such as gastrointestinal protective, anti-bacterial, anti-oxidant, anti-cancer and anti-inflammatory activities. The main active components are present in the Patchouli essential oil (PEO) extracted fractions. These include *patchouli alcohol* (PA), *β-patchoulene* (β-PAE), and *pogostone* (PO). In this study, we hypothesize that PEO and the pure active compounds might interact with gut residing bacteria and yield beneficial effects to the host.

In this study, 25 C57BL/6J mice were equally divided into five groups and gavage separately with non-toxic doses. The fecal microbial DNA were analyzed by the ERIC-PCR and 16S rDNA amplicon sequencing.ERIC-PCR analysis distinctly clustered microbial composition in control and treated mice.

Using 16S amplicon sequencing, we noticed improved OTUs diversity in mice treated with PA, β-PAE. Through weighted uniFrac analysis, we identified that the abundant composition of gut microbiota is far distinct in mice treated with PA compared to control mice. Furthermore, the abundance of phylum Firmicutes elevated in all the treated mice. Whereas, marked decline was noticed in the abundance of phylum Verrucomicrobia which is mainly related to the inhibited growth of genus Akkermansia. Through LEfSe analysis, we identified differential bacterial families that were specific to each group; the improved growth of Bifidobacteriacea and Burkholderiaceae in β-PAE treated mice; and promoted growth of Prevotellaceae and Mycoplasmataceae in PO treated mice.

We conclude that the PC extracts and the purified compound have the potential to modulate gut microbial composition. Comparatively, we noticed promoted growth of probiotic bacteria and suppressed abundance of potential pathogen in mice treated with β-PAE and PO.

S35-0001

Mode of Action of Surface Bound Antimicrobial Peptides melimine and Mel4 against Pseudomonas aeruginosa

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Biomaterial associated infections are a multi-billion-dollar burden globally. Antimicrobial peptide-based coatings may be able to prevent such infections. The aim of this study was to investigate the mechanism of action surface bound peptides (AMPs) against Pseudomonas aeruginosa 6294. Melimine and Mel4 were covalently attached to glass cover slips using azido-benzoic acid. Attachment was confirmed using X-ray photoelectron spectroscopy. P. aeruginosa was allowed to attach to AMP-coated glass for up to 6 hours. The effect of the surface bound AMPs on bacterial cell membranes was evaluated using the dyes DiSC3-(5), Sytox green, SYTO 9 and propidium iodide with fluorescence microscopy. Release of cytoplasmic materials ATP and DNA/RNA were determined in the surrounding fluid. The amount of cell death was estimated by agar plate counts. The AMPs were successfully covalently bound to the glass as demonstrated by increases in %nitrogen of 5.4% (melimine) and 4.8% (Mel4) compared to controls. Immobilized peptides disrupted the cytoplasmic membrane potential of P. aeruginosa within 10 min. This was followed by release of ATP after 2 h. Membrane permeabilization started at 3 h of contact with glass coated AMPs. There was a significant number of bacteria (59% for melimine; 36% for Mel-4) with damaged membranes after 4 h of contact. At the 6 h time point, release of DNA occurred with melimine releasing 2 times the amount of DNA/RNA than Mel4 surfaces ($p<0.05$). Surface bound AMPs were able to disrupt cell membranes with subsequent release of cytoplasmic materials, and ultimately resulting in bacterial death.

S35-0002

The epidemiology of extended-spectrum β-lactamase-producing (ESBL) infections in a tertiary teaching hospital in China from 2015-2018

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Aim: To understand the epidemiology and characteristics of the extended-spectrum β-lactamase-producing (ESBL) infections in hospital in order to find effective interventions to reduce antibiotic resistance and strengthen antibiotic stewardship.

Methods: Cross-sectional design to collect data from the hospital information management system from 1 January 2015 to 30 August 2018. Variables included patient age, sex, diagnosis, department and antibiotic sensitivity test results.

Results: In total 4748 samples were collected from 1166 patients from 2015-2018. The mean age was 62 years, 60% of them were > 66 years. More than half of the samples were from internal medicine inpatients. 56% of patients were male. 38% of the total samples were from urine, 25% from sputum, followed by secretion, drainage, body fluid and catheter. Among the total sample, antibiotic resistance rates were 44%. Extended-spectrum beta-lactamase (ESBL) prevalence was 6.4% with seasonal trends. Two peaks were seen in May/June and August/September each year. The top 6 ICD-10 diagnoses associated with resistance were: hypertension, brain diseases, appendicitis, pneumonia, not specified diagnosis, diabetes and urinary tract infections. The antibiotic resistance rankings were cefotaxime,

ceftriaxone, aztreonam, ceftazidime. The rates of resistance to antibiotics declined from 2015 to 2018 with average rates of 1.7%, 1.4%, 1.0% to 1.1%. Logistical regression showed that being a female, age 80-102, emergency department visit, diagnosis with appendicitis, kidney diseases, pneumonia and diabetes were associated with ESBL infections after adjustment for other potential confounders.

Conclusion: ESBL infection rates were not high in this tertiary hospital. However, with the implementation of antibiotic stewardship monitoring since June 2016 in this hospital, there was sharp decline with a slight rise thereafter. The policy will need more focus in the emergency and ICU departments.

S35-0003

Pharmacodynamics and safety evaluation of a new Antimalarial, HP6H8, a humanized CD147 antibody

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Malaria remains a deadly epidemic infectious disease. Our previous study published on *Blood* have demonstrated that the CD147-RAP2 interaction is essential for erythrocyte invasion by *Plasmodium falciparumand* and a humanized CD147 antibody, HP6H8, which specifically interrupts the CD147-RAP2 pair, is capable of complete elimination and prevention of P falciparum infection in humanized mice. Here, we conducted the further pharmacodynamics and safety evaluation of the antibody. Interestingly, HP6H8 showed superior cure and preventive functions on Dd2 strain compare to Chloroquine in humanized mouse model. In single- and repeat-dose toxicology studies in Rhesus monkeys, HP6H8 did not induce any distinct or novel adverse findings and was well tolerated at all tested doses. These preclinical safety data facilitated the initiation of an ongoing clinical trial of HP6H8.

S36-0001

Linking Non-canonical Inflammasome Activation and Endotoxic Shock in Zebrafish Model

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The non-canonical inflammasome is critical for cytosolic sensing of gram-negative bacteria in the innate immune responses. However, the mechanism of bacterial factors modulating non-canonical inflammasome activation and altering immune defenses or endotoxic shock remains to be investigated. In our work, a fish pathogen *Edwardsiella piscicida* mutant, *0909I*, was identified to induce a robust caspase-1-independent pyroptotic-like cell death via overexpressed-hemolysin in zebrafish non-phagocyte cells. Moreover,the hemolysin was found to largely associate with bacterial outer membrane vesicles (OMVs), which were internalized into cells via a dynamin-dependent endocytosis, then promotes LPS release into cytosol of fish cells to induce the pyroptotic-like cell death. Furthermore, we revealed that this pyroptosis requires Caspy2 activation in zebrafish, and interestingly, Caspy2 could directly bind to lipopolysaccharide via its N-terminal pyrin death domain, resulting into oligomerization. Consequently, we found that Caspy2 is highly expressed in the zebrafish gut, and essentially, knockdown of *caspy2* expression impaired the ability of zebrafish to restrict bacterial invasion *in vivo*, and also protected larvae from endotoxic shock. Taken together, our results not only identify a crucial event in the evolution of pattern recognition into the death domain superfamily-mediated intracellular lipopolysaccharide-sensing pathway in innate immunity, but also reveal a unique model to study the impact of noncanonical inflammasome activation and endotoxic shock progression with clear complementarities with the mouse model, which could lead to the possibility of genetic and chemical screenings in a whole organism.

S36-0002

Regulation of pyocyanin biosynthesis by transcriptional regulator PsrA in Pseudomonas aeruginosa PAO1

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Pseudomonas aeruginosa is a common pathogen that causes nosocomial infections. It produces a blue-green pigment that called as pyocyanin, which is one of the major virulence factors in *P. aeruginosa*. The pigment is known to disrupt host cells by causing oxidative stress. The biosynthesis of pyocyanin is controlled by quorum sensing which is also regulated by PsrA.

PsrA is a transcriptional regulator that regulates the expression of many physiological and virulent-related genes in *P. aeruginosa*. The aim of this study is to investigate the mechanism of pyocyanin biosynthesis regulation via PsrA in *P. aeruginosa*. Initially a transcriptome analysis was performed to compare the expression level of pyocyanin biosynthesis-related genes of wild-type PAO1 with that of *psrA* overexpression strain. Then, the quantity of pyocyanin, quorum sensing autoinducer, exopolysaccharide, and hemolytic activity were determined in the wild-type, *psrA* overexpression and *psrA* deletion strains.

The results showed that in *psrA* overexpression strain: 1) the expression of *phz* operon, responsible for pyocyanin synthesis, increased compared with that of the wild-type PAO1; 2) the expression of AHL quorum sensing system genes was reduced, and also less AHL autoinducer was produced than in wild-type PAO1; 3) the expression of quinolone quorum sensing system (Pqs)-related genes which was positively regulated by LasR was increased; 4) the activity of hydrolytic enzymes, including elastase, hemolysin, caseinase, were lower than wild-type PAO1, so was the expression of their encoding genes. Electrophoretic mobility shift assay (EMSA) was performed to locate PsrA binding site at 1 to 250-bp upstream of start codon of *lasR*. Altogether, these findings will contribute to comprehensive understanding of how PsrA regulates pyocyanin biosynthesis and the importance of PsrA on physiology and pathogenesis in *P. aeruginosa*.

S36-0003

PB1-F2 protein of highly pathogenic avian influenza A (H7N9) virus suppresses MAVS-mediated antiviral responses through enhancing MAVS degradation

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PB1-F2 protein is the 11th influenza A viral protein discovered in 2001. It was reported to be a pathogenic marker to IAV infection with unclear mechanism. Though it was reported to be pro-apoptotic to kill immune cells, the pro-apoptotic function was found irrelevant to most pathogenic IAV strains at later studies. More recently, studies suggested that PB1-F2 of pathogenic IAV strains suppresses early antiviral responses to mediate IAV pathogenesis by PB1-F2 N66S mutation. Mechanistic studies showed that N66S mutation enhanced PB1-F2-mediated mitochondrial membrane potential ($\Delta\Psi m$) dissipation and the strength of binding to MAVS protein, a central adaptor protein of antiviral responses against IAV infection. However, N66S pathogenic mutation is not common to all human pathogenic IAVs. In the current study, the PB1-F2 protein of human pathogenic influenza A (H7N9) virus was analyzed. We found that though H7N9 PB1-F2 did not carry N66S mutation, it potently suppressed MAVS-mediated antiviral responses at MAVS level but not at downstream effectors. We thus hypothesized that H7N9 PB1-F2 affects MAVS protein with novel mechanism independent to N66S mutation. Indeed, H7N9 PB1-F2 was neither tightly bound to MAVS protein nor dissipating $\Delta\Psi m$. Instead, H7N9 PB1-F2 protein reduced MAVS protein level under Sendai virus infection or MAVS overexpression. Cycloheximide chase assay showed that H7N9 PB1-F2 enhanced MAVS protein degradation. It was further showed that H7N9 PB1-F2 targeted MAVS aggregate for degradation that reduced MAVS signalosome formation. The H7N9 PB1-F2 mediated-immunosuppressive effect and MAVS degradation were also observed when recombinant IAV expressing H7N9 PB1-F2 was assayed through human macrophages, the primary immune cells against IAV infection. Taken together, we propose that H7N9 PB1-F2 is a potent suppressor against MAVS-mediated antiviral responses by enhancing MAVS degradation

S36-0004

Identification of CSNK2A2 loci associated with systemic lupus erythematosus (SLE) and evaluation of SLE genetics in drug repositioning

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Systemic lupus erythematosus (SLE) is prototype autoimmune disease with a diverse spectrum of clinical symptoms, ranging from skin rash to end-organ damage. Through trans-ethnic genome-wide association studies (GWAS), we identified a novel SLE-associated locus that passed the genome-wide significance, *CSNK2A2* (rs2731783, *P*meta = 1.08E-09). We further performed fine mapping analyses at the newly identified locus and found two putative causal variants. The putative casual variants reside in an enhancer and are associated with expression of *CSNK2A2* in B-lymphocytes, suggesting a potential mechanism of the association. To explore biological insight underlying the SLE susceptibility loci, we prioritized putative risk genes by integrating the GWAS findings with functional annotations. We identified 78 putative SLE risk genes and demonstrated that they are more likely to encode proteins interacting with molecular targets of approved SLE drugs. Our study suggested that several drugs approved for other diseases might have effects on SLE patients. In summary, this study identified a novel locus associated with SLE and demonstrated the role of SLE GWAS findings in drug repositioning.

S36-0005

Modulation of Gut Microbiome for Preventing and Treating Rheumatoid Arthritis

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Gut microbial dysbiosis is linked to several immune-mediated diseases, including rheumatoid arthritis (RA). Modulating aberrant microbiotas into a healthy state is a potential therapeutic approach for treating autoimmune diseases. Here, we detected for the first time dynamic alterations of the gut microbiome using metagenomic shotgun sequencing in an RA animal model using rats treated with *Lactobacillus casei (L. casei)* as a preventative intervention. Based on our newly established catalog of the gut metagenome in Sprague-Dawley (SD) rats, dysbiosis in the microbiome of arthritic rats was partially resolved after *L. casei* intervention, and the restored microbial species were closely correlated with bone protection and cytokine regulation, as determined by this metagenome-wide association study. Additionally, the microbial species in the common effective medical interventions showed similar alterations to *L. casei*, and the microbial changes in these treatments affected functional modules by regulating the transport system and metabolism pathways. These results indicate that preventive intervention using *L. casei* with other common anti-arthritis medications redirects the gut microbiome to a healthy state, suggesting that modulating the microbiota may be a potential approach for arthritis treatment. (Acknowledgments: This work was financially supported by grants from the Macau Technology Development Fund 102/2016/A3, FDCT-17-029-SKL)

S36-0006

Is the airway microbiome a link between rheumatoid arthritis and interstitial lung disease?

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Interstitial lung disease (ILD) is a critical extra-articular feature of rheumatoid arthritis (RA) and a major cause of mortality. The underlying pathogenesis of RA associated ILD (RA-ILD) remains unknown, but increasing evidences reveal a potential role of lung commensal microbiome on inducing aberrant immune response which finally contributes to the occurrence of ILD in genetically susceptible individuals. Airway dysbiosis may exert its promoting effects on both RA and RA-ILD *via* anti-citrullinated peptide antibody (ACPA). Higher ACPA-IgA prevalent suggests a persistent mucosal immune inflammation in RA-ILD and Idiopathic pulmonary fibrosis (IPF) rather than non-ILD RA subjects. An elevation in ACPA-IgG triggers to the onset of rheumatoid arthritis in IPF subject with ACPA serum positivity. Additionally, airway microbiome can also affect the occurrence and development of RA-ILD through the following ways. Firstly, microbiome was recognized as antigen inducer of rheumatoid arthritis specific antibody, such as citrullinated peptide to ACPA by bacterial peptidyl arginine deiminase (PAD), or host PAD from Netosis. Secondly, microbiome acts as inflammation regulator, which limits inflammation and harmless antigens, as well as maintains the balance between the pro-inflammation and the anti-inflammation bacteria. Besides, the composition of microbiome could be regulated, as determined by the ecological niche to pathogenic microbiota, e.g. *Fusobacterium nucleatum* promoting *Porphyromonas gingivalis* colonization in pristine induced arthritis (PIA) model. This review provides an overview of the effects of airway microbiome on the pathogenesis and development of RA-ILD focusing on microbiome-mucosal immune crosstalk and suggests that ACPA could be used as a therapeutic target for RA-ILD. (Acknowledgements: This work was financially supported by grants from the Macau Science and Technology Development Fund 102/2016/A3, FDCT-17-029-SKL).

S38-0001

Alternative strategies to tackle antibiotics resistance

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The emergence of multidrug resistant bacteria including methicillin resistant Staphylococcus aureus (MRSA) is the inevitable outcome of indiscriminate use of antibiotics by mankind. As the World Health Organization has declared antimicrobial resistance one of the biggest threats to global health, alternate therapeutic strategies that will not cause resistance are urgently needed to fight against MDR bacteria.
Two strategies, restore the sensitivity of resistant bacteria to known antibiotics and suppress the virulence of bacteria. A known drug colloidal bismuth subcitrate, can repurpose meropenem to the Enterobacteriaceae producing metallo-β-lactamases, such as NDM-1 *E. coli*. The unique mechanism is one Bi(III) displacing two Zn(II) ions as revealed by X-ray crystallography, leading to the release of Zn(II) cofactors. Two novel compounds could reduce MRSA virulence through different ways. NP16 reduce the "shield" of MRSA, staphyloxancin, and increased the sensitivity of bacteria to our immune system; M21 disarms MRSA by suppressing multiple virulence factors production and reduces the pathogenicity of bacteria. They all showed promising effect against pathogens in vivo.
Since this type of compounds have two advantages over classic antibiotics. Firstly, such agents will not have selective pressure for raising resistance mutant. Secondly, the specificity of such agents should preserve the bacteria that constitute the normal flora. It is anticipated that they will become a new therapeutic strategies.

S38-0002

Chrysosplenetin reverses artemisinin resistance by down-regulating P-glycoprotein (P-gp)/Mdr1a (ABCB1) mRNA levels but not breast cancer resistance protein (Bcrp) expression in mice small intestine post-infected by artemsinin-sensitive or -resistant rodent parasites Plasmodium berghei K173

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In this study, we aimed to verify if the mechanism of chrysosplenetin on reversing artemisinin resistance in artemisinin-sensitive or -resistant Plasmodium berghei K173 infected mice. Infection with sensitive or resistant malarial parasites led to a turbulent mice body weight, injured small intestines, and lower percent survival while artemisinin and artemisinin-chrysosplenetin combination, in some degree, alleviated it. Negative control and novobiocin did not indicate an anti-malarial effect whether on the sensitive or resistant malaria parasites infected mice. Interestingly, verapamil and chrysosplentin in the presence and absence of artemisinin also did not display an obvious efficacy on sensitive P. berghei K173 while showed a strong anti-malarial effect on resistant parasites infected mice. Artemisinin activated Mdr1a/ABCG2 mRNA expression and chrysosplenetin-artemisinin combination down-regulated the levels. Also, they reversed the up-regulated expressed P-gp protein levels induced by artemisinin.

Oppositely, artemisinin decreased Bcrp expression while chrysosplenetin with or without artemisinin significantly increased it. Chrysosplenetin, therefore, is considered to reverse artemisinin resistance due to the inhibition effect on P-gp/Mdr1a mRNA but not Bcrp. Our data provided a possible proof that some P-gp inhibitors without anti-malarial effect might be effective to the artemisinin-resistant P. berghei instead.

S41-0001

Synergistic combination of benzyl isothiocyanate and caffein boosted the death of human breast cancer cells

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Background: Benzyl isothiocyanate (BITC) have been identified as potential potent anticancer agent found in Brassicales plant. Our present study demonstrates the trends and mechanisms of cytotoxicity effects of BITC, caffeine and the novel combination of BITC and caffeine to enhance the effectiveness of the cancer treatment.

Methods: Cytotoxicity effects of BITC, caffeine and their combination toward human breast cancer (MCF-7) cells were evaluated by using MTT assay after 24 and 48 hours of treatments. We explored mechanisms of cytotoxicity effects by examining the protein expression of p38 MAPK, ERK 1/2, Nrf-2, Bcl-2 and glutathione-S-transferase (GST) by Western blot technique. Glutathione (GSH), oxidative stress and caspase 3/7 level were measured fluorometrically.

Results: BITC inhibits the growth of MCF-7 cells. However, caffeine was not toxic towards MCF-7 cells. Interestingly, we found that the combination of BITC and caffeine created the synergistic effect to kill MCF-7 cells in time and dose-dependent. BITC increased the oxidative stress level of MCF-7 cells as a mechanism to induce the cell death. Distinctively, oxidative stress level decreased when caffeine combined with BITC. Activation of MAPK pathway by the up-regulation of p38, ERK1/2, NRF-2 induced MCF-7 cells death after the treatments. Our results revealed the up-regulation of GST correlated with the depletion of GSH of the treated MCF-7 cells. Furthermore, the elevation of caspase 3/7 and activation of Bcl-2 indicated apoptotic death mechanism of MCF-7 cells death.

Conclusion: Taken together, caffeine boosted the efficacy of BITC to kill the cancer cells by activating MAPK pathway. The elevation of GST catalyzed the conjugation of GSH with BITC and caffeine thus lowered the amount of GSH and caused the death of cancer cells by the imbalance redox homeostasis. Synergistic combination of bioactive compounds from edible sources potentially makes the cancer treatments safer and cheaper.

S41-0002

Exome sequencing combined with translatome analysis of colon polyps to identify the actionable biomarkers for clinical diagnosis

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The incidence rate of colorectal cancer is the highest among the cancers in Taiwan. Survival for colon carcinoma closely relates to the clinical and pathologic stage of the disease at diagnosis. Therefore, if the disease can be detected at an early stage, overall prognosis can be improved. The development of colorectal cancer is start from the proliferation of epithelial cell, polyps formation and finally becomes tumor. Therefore, we are interesting to study the mechanism which induces the colon polyps formation and finally become colorectal cancer.

Two kinds of whole genome analysis technologies, exome sequencing and ribo-seq, were used to dissect the molecular mechanism of polyps progressing. From the exome sequencing result, the CNV was found on the tubular polyps and villous polyps, but only small genome regions amplification or deletion were found in sessile serrated polyps(SSA). Several SNPs to distinguish the subtype of polyps were also be discovered.

Ribosome profiling (ribo-seq) is a new technique that provide the genome-wide information on protein synthesis in vivo. This technology already dramatically change our understanding of translational control. In my lab, we have successfully established this technology and data analysis pipeline to get a more high quality data with lower cost. After analyzing 4 HPs and 7 SSA/Ps samples by principal components analysis and hierarchical clustering, we identify 6 genes to be potential markers to distinguish SSA/Ps from HPs. Overall, we identity several good biomarkers for further validation and highlight the role of Ribo-Seq technology in biomarker discovery.

S41-0003

The transcriptional landscape of B-cell precursor acute lymphoblastic leukemia based on an international study of 1,223 cases

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Most B-cell precursor acute lymphoblastic leukemia (BCP ALL) can be classified into known major genetic subtypes, while a substantial proportion of BCP ALL remains poorly characterized in relation to their underlying genomic abnormalities. We therefore initiated a large-scale international study to reanalyze and delineate the transcriptome landscape of 1,223 BCP ALL cases using RNA-seq. Fourteen BCP ALL gene expression subgroups (G1-G14) were identified. Apart from extending eight previously described subgroups (G1-G8 associated with *MEF2D* fusions, *TCF3-PBX1* fusions, *ETV6-RUNX1* positive/*ETV6-RUNX1*-like, *DUX4* fusions, *ZNF384* fusions, *BCR-ABL1*/Ph-like, high hyperdiploidy and *KMT2A* fusions), we defined six additional gene expression subgroups: G9 was associated with both *PAX5* and *CRLF2* fusions; G10 and G11, with mutations in *PAX5* (p.P80R) and *IKZF1* (p.N159Y), respectively; G12 with *IGH-CEBPE* fusion and mutations in *ZEB2* (p.H1038R), and G13 and G14, with *TCF3/4-HLF* and *NUTM1* fusions, respectively. In pediatric BCP ALL, subgroups G2-G5 and G7 (51-65/67 chromosomes) were associated with a low risk, G7 (with ≤50 chromosomes) and G9 were intermediate risk, whereas G1, G6 and G8 defined high risk subgroups. In adult BCP ALL, G1, G2, G6 and G8 were associated with high risk, while G4, G5 and G7 had relatively favorable outcomes. This large-scale transcriptome sequence analysis of BCP ALL revealed distinct molecular subgroups that reflect discrete pathways of BCP ALL, informing disease classification and prognostic stratification. The combined results strongly advocate that RNA-seq is introduced into the diagnostic workup of BCP ALL within the clinical setting.

S41-0004

Using predictive analytics to optimize the performance of fecal immunochemical test screening for colorectal cancer

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Fecal immunochemical test (FIT) has been increasingly adopted by colorectal cancer screening programs in multiple countries over recent decades. Screenees would typically be referred for colonoscopy if their FIT values exceed a pre-defined positivity threshold (e.g. 100 ng/ml in Taiwan). These triage algorithms do not necessarily account for current local epidemiology of colorectal cancer (e.g. prevalence of the different disease stages in the natural history of colorectal cancer). We hypothesize that FIT screening can be optimized by supplementing an individual's FIT value with current local epidemiology of colorectal cancer to predict her risk of advanced neoplasia (i.e. by answering the question "What is the probability of advanced neoplasia given the individual's FIT value and current local epidemiology of colorectal cancer). Using data from 66,697 participants of the Hong Kong Colorectal Cancer Screening Pilot Program which was launched in Sep 2016, we developed a model with such predictive function and used decision curve analysis to show that it provides higher clinical value that the status quo threshold-based triage algorithm. The model can be easily implemented in the existing program and updated with new screening data in real-time.

S41-0005

MMP-9 targeted by miR-494 promotes silybin-inhibited osteosarcoma

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Osteosarcoma (OS) is the most common malignant tumor that develops in bone. Its mortality is very high. Therefore, study of mechanisms of pathogenesis of the OS is urgently required. Previous studies of microarray showed that the expression levels of matrix metallopeptidase 9 (MMP-9) altered significantly in OS. In addition, overexpression of MMP-9 is recognized as an indicator in cancer. However, the exact roles of MMP-9 in OS are not fully investigated. Thus, we firstly studied the roles of MMP-9 in OS and revealed that silence of MMP-9 inhibited OS cell proliferation as determined by MTT assay and colony formation assay. Secondly, we conducted TUNEL assay and confirmed loss of functions of MMP-9 induced OS cell apoptosis. Next, we used lentivector packaging method to overexpress MMP-9 and found that overexpression of MMP-9 promoted OS cell migration. Fourthly, the results of luciferase assay showed that MMP-9 was targeted by hsa-miR-494, which inhibited OS. Fifthly, we revealed that the levels of hsa-miR-494 were upregulated by the drug silybin which inhibited OS. Finally, we revealed that silybin inhibited OS cell viability by altering the protein levels of β-catenin and Runt-related transcription factor 2 (RUNX2) as determined by western blot and immunocytochemistry (ICC).

S41-0006

Herbal formula YNHY inhibits human malignant glioma through induction of caspase-mediated apoptosis

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Glioblastoma (GBM) is the most common and aggressive primary brain tumor in people. In searching for herbal formulas with anti-cancer activities from Traditional Chinese Medicines, we found YNHY

was of great interest. Our results showed that YNHY inhibited cell proliferation of human malignant glioma cell line U87-MG in a dose- and time-dependent manner, which was further confirmed by colony formation assay. It induced cellular apoptosis through initiation of caspase-mediated signaling pathway as analyzed by flow cytometry and western blot. Furthermore, YNHY significantly inhibited xenograft growth of U87-MG cells in nude mice. Our findings suggest that YNHY exerted a profound anti-tumor effect and induced apoptosis in U87 cells. (This work was supported by grant from the Science and Technology Development Fund of Macau, project code: 034/2015/A1)

S41-0007

Functional and Mechanistic Characterization of PRMT6-Regulated Autophagy in Hepatocellular Carcinoma

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Autophagy is a critical survival factor for cancer cells, whereby it maintains cellular homeostasis including degradation of damaged organelles and unwanted proteins as well as the support of cellular biosynthesis in response to environmental stress, preventing cells from undergoing apoptosis. This study investigates on the role of autophagy in cancer development, in the context of the Asian prevalent tumor type, hepatocellular carcinoma (HCC). HCC is a major health burden worldwide. Tumor recurrence and therapy resistance represent major obstacles in the treatment of the disease, with their mechanism of action largely uncharacterized. Our group has recently reported on the role of protein arginine methyltransferase 6 (PRMT6) down-regulation in potentiating HCC resistance to sorafenib and chemotherapy (Chan et al. *Cell Rep* 2018; accepted). Upon autophagy induction by various stimuli including sorafenib (stress inducer), EBSS (nutrient deprivation) and hypoxia, we found autophagic flux in HCC cells to be negatively correlated with PRMT6 expression. Intriguingly, a number of autophagy-related proteins were identified as potential PRMT6-binding partners by mass spectrometry-based proteomics. Mechanistically, PRMT6 is a well-known enzyme that plays an important role in post-translational modifications via arginine methylation. In this study, we demonstrated PRMT6 to bind to and methylate its binding partners on arginine residues and as a result altering its downstream signaling pathway eventually leading to its protein instability and localisation. Our findings suggest PRMT6 down-regulation in HCC tumors to promote tumorigenicity and sorafenib resistance through autophagic flux de-regulation and anti-apoptotic activity. Targeting this mechanism of stress response may provide novel therapeutic insights for this deadly disease.

S41-0008

Phosphoserine phosphatase (PSPH) is up-regulated in glioma and predicts poor survival of glioma patients

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Glioma is the most common malignant tumor in the central nervous system. Although advances have been made in surgery and adjuvant therapies in recent years, the median survival of glioma patients remains almost unchanged. The inability to cure this disease lies in uncontrolled proliferation, migration and invasion. Recent research attention has been redrawn to the field of cancer metabolism, of which the serine synthesis pathway (SSP) is believed to critical for cancer cell proliferation. However, the roles of SSP in glioma have not been elucidated so far. We firstly analyzed the public database (GEPIA) and found that the mRNA levels of the three important enzymes of this pathway: phosphoglycerate dehydrogenase (PHGDH), phosphoserine aminotransferase 1 (PSAT1) and phosphoserine phosphatase (PSPH) were all up-regulated in glioma. Of note among those enzymes, PSPH showed a grade-dependent increase in glioma tissues and predicted poor overall and disease-free survival of glioma patients. What's more, the cBioPortal database showed that PSPH bore the highest amplification percentage (6%) compared to the other two enzymes (both < 1%) in 794 glioma tissues. These results were then verified with clinical specimens collected from our hospital by quantitative real-time polymerase chain reaction (q-PCR), immunohistochemistry (IHC) and western blot. These findings suggest that PSPH is upregulated in glioma and could serve as a prognostic biomarker for glioma.

S42-0001

Contrasting effects of host species and phylogenetic diversity on the occurrence of HPAI H5N1 in European wild birds

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Studies on the highly pathogenic avian influenza (HPAI) H5N1 suggest that wild bird migration may facilitate its long-distance spread, however the role of wild bird community composition in its transmission risk remains poorly understood. In addition, most studies on the diversity–disease relationship focused on host species diversity without considering hosts' phylogenetic relationships, which may lead to rejection of the effect of species diversity when the community has host species that are only distantly related. We here explored the role of the waterbird community composition in determining HPAI H5N1 occurrence in wild birds in a continental-scale study cross Europe. We particularly tested the diversity–disease relationship using both host species diversity and phylogenetic diversity. By this continental-scale study, we here provide the first demonstration that host community composition, compared to previously identified environmental risk factors, can also effectively explain the spatial pattern of H5N1 occurrence in wild birds. We further show that communities with more species of higher–risk hosts and more closely related species had a higher risk of H5N1 outbreaks. Thus, host species diversity, community phylogenetic structure and environmental factors jointly influence H5N1 occurrence. Our work not only extends current theory on the diversity–disease relationship, but also has important implications for future surveillance of H5N1 and other HPAI subtypes.

S45-0001

Characterization of the regulation mediated by conserved peptide uORFs in the key regulators of Glucosinolates biogenesis

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Aliphatic, indolic and benzyl glucosinolates (GSLs) are sulfur-containing secondary metabolites found in plants of the Brassicale order and play important roles in defense against herbivores and pathogens. The biosynthesis of each class of GSLs is regulated by distinct *MYB* transcription factors, some of which harbor conserved peptide upstream open reading frames (CPuORFs). We have previously shown that indolic *MYB34* and *MYB51* CPuORFs in Arabidopsis possess the footprints of stacked ribosomes and repress downstream main ORF expression. Here, using protoplast transient assays, we further showed that papaya benzoyl *MYB* CPuORF also down-regulates downstream main ORF expression as Arabidopsis indolic *MYB*CPuORFs. However, aliphatic *MYB28* and *MYB29* CPuORFs in Arabidopsis lack the footprints of stacked ribosome and confer up-regulation of downstream main ORF unexpectedly. Interestingly, Arabidopsis indolic *MYB51* CPuORF acts as a positive regulatory element in a mutant defective in the first step of indolic GSL core structure biosynthesis while remaining a negative regulator in a mutant defective in the final step of this pathway. This result suggests that indolic GSL intermediates might be involved in the regulatory function of Arabidopsis indolic *MYB51* CPuORF. Taken together, our results indicate that the function of GSL *MYB* CPuORFs is diversified during evolution.

S46-0001

The role of MT1-MMP in the regulation of glucose metabolism and body weight

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Obesity-associated insulin resistance is a hallmark of type 2 diabetes mellitus and plays a central role in metabolic syndrome. Recent studies showed that mutations in MMP14, a membrane bound metalloproteinase, have been associated with human obesity and diabetes traits. Despite the genetic association between MMP14 gene polymorphisms and obesity traits, the molecular mechanism by which MT1-MMP regulates insulin sensitivty remains elucidated. We hereby reported that the expression of MT1-MMP is elevated in both obese and aged mice. Heterzygous loss of MT1-MMP improved glucose tolerance with enhanced systemic insulin sensitivty in mice on high fat diet, as well as in aged lean 1-year-old chow-diet fed mice. Mechanically, MT1-MMP cleaves the insulin receptor (IR) and inhibits downstream IR signaling. These observations reveal a novel role for MT1-MMP in the regulation of insulin resistance and suggest that inhibition of MT1-MMP could be a therapeutic strategy for diabetes treatment.

S46-0002

Transcriptome and functional analysis of Beige adipocytes induced from intra-abdominal and subcutaneous white adipose tissues

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Beige adipocytes can be induced from white adipocytes and precursors upon stimulation by cold temperatures and act like brown adipocytes to increase energy expenditure. Most in vivo studies examining the mechanisms for the induction of beige adipocytes have focused on subcutaneous white adipose tissue (sWAT; benign fat) in the mouse. How intra-abdominal WAT (aWAT; malignant fat) develops into beige adipocytes remains obscure, largely because there is a lack of a good animal model for the induction of beige adipocytes from aWAT. To better understand the development of beige adipocytes from mammalian WATs, especially aWAT, we induced beige adipocytes from bat aWAT and mouse sWAT by exposure to cold temperatures and analyzed their molecular signatures. RNA sequencing followed by whole genome-wide expression analysis shows that beige adipocytes induced from bat aWAT, rather than sWAT, have molecular signatures resembling those of mouse sWAT-induced beige adipocytes and exhibit dynamic profiles similar to those of classical brown adipocytes. In addition, we identified molecular markers that were highly enriched in beige adipocytes and conserved between bat aWAT and mouse sWAT, a set that included the genes *Uqcrc1* and *Letm1*. Furthermore, knockdown of *Uqcrc1* and *Letm1* expression shows that they are required not only for beige adipocyte differentiation but also for preadipocyte maturation. This study presents a new model for research into the induction of beige adipocytes from aWAT in vivo, which, when combined with models where beige adipocytes are induced from sWAT, provides insight into therapeutic approaches for combating obesity-related diseases in humans.

S46-0003

PKM2: a key enzyme for regulating cancer metabolism

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Pyruvate kinase M2 (PKM2), a key enzyme in Warburg effect, is an exclusive exon-10 splice isoform from the *PKM* gene. Pyruvate kinase activity of PKM2 is modulated through allosteric regulation and post-translational modifications, which can divert mitochondrial oxidative phosphorylation in favor of aerobic glycolysis. PKM2 also partners with KDM8, an oncogenic demethylase, which translocate into the nucleus to serve as a transcriptional co-activator of HIF1α contributing to the Warburg metabolism. Highly abundant and co-occurrence of PKM2 and KDM8 is often found in breast cancer. Here, we present evidence of the mechanistic foundation by which PKM2 plays a key role in regulating ATP production and biosynthetic processes. As such, PKM2 acts as an integrator to meet the metabolic requirements of proliferating and non-proliferating cells. Targeting PKM2-mediated pathway provides unique opportunities for novel cancer diagnosis and therapeutics.

S46-0004

Synergistic effect of combination of statins and 2-dexoyglucose on growth inhibition of human colon cancer cells

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Targeting cancer cell metabolism is a strategy to treat cancer. Statins, HMG-CoA reductase inhibitors with potent lipid-lowering effects, are widely used in prevention and treatment of cardiovascular diseases. Recent studies have been proposed the antitumoral and antiproliferative activities of statins. 2-deoxy-D-glucose (2DG) is a glycolytic inhibitor resulting in energy starvation. However, anti-cancer effect of statins in combination with 2DG has not been evaluated. Here, we examined the effect of statins in combination with 2DG against human colon cancer cells. The combination of lovastatin and 2DG was much more harmful for cancer cells than the treatment with lovastatin or 2DG alone, leading to 91.43% inhibition of cell viability comparing to 77.18% and 72.21% respectively when lovastatin and 2DG used alone. The combination also significantly inhibited the colony formation and induced of apoptosis than either drug used alone. Almost all combination Index (CI) values, calculated by CompuSyn software, were<1, suggesting that the combination of statin and 2DG was synergistic rather than additive against colon cancer cells. In vivo, the combination treatment significantly inhibited the tumor growth in HCT116 tumor xenograft nude mice. Hence, our study reinforces the value of metabolic reprogramming in cancer treatment and suggests that the combination of statins and 2DG may present a promising anti-cancer therapy. (This work was supported by grant from the Science and Technology Development Fund of Macau, project code: 034/2015/A1)

S47-0001

Whole genome non-coding RNA profiling of the Down syndrome developing hippocampus using the mouse model Dp16(1)Yey

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Down syndrome (DS), caused by the trisomy of chromosome 21 (HSA21), is the most frequent human chromosomal disorder. Hippocampal-dependent learning and memory impairment is one of the most significant deficits of DS. Non-coding RNAs (ncRNA) have been increasingly revealed to be participating in the brain development and neuronal functions. In this study we performed a systematic analysis of the ncRNA transcriptomes of the developing hippocampus using the DS mouse model *Dp16(1)Yey*. The miRNA, lncRNA, and circRNA profilings were conducted using the next-generation sequencing method. 93 miRNAs were found significantly dysregulated in the *Dp16(1)Yey* developing hippocampus, among which only miR-155 was derived from the triplicated genomic region. Co-analysis of the differentially expressed mRNAs and miRNAs highlighted the regulation of *miR-204/miR-211* on *Arhgap11a* which could regulate the cell proliferation and differentiation. Total of 80 lncRNAs were found significantly dysregulated. Co-analysis of the differentially expressed lncRNAs and miRNAs identified an interaction map cored with lncRNAs LOC102633000 and Gm42135. Unfortunately, we were unable to find the human orthologues for the differentially expressed lncRNAs. Total of 1522 circRNAs were found differentially expressed. KEGG pathway analysis highlighted the GABAergic synapse pathway. Our results indicate that dysregulated circRNAs may play roles in the etiology of excessive GABAergic inhibition observed in the DS hippocampus, while dysregulated miRNAs may participate in the proliferation of neuron progenitors which may result in the hypocellular observed in the DS hippocampus. In contrast, DS mouse models may be not good options for the DS lncRNA studies due to the low conservation of the lncRNA sequences.

S47-0002

The regulation role of histone deacetylase 5 in peripheral neuropathic pain

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Neuropathic pain has a considerable impact on the quality of patients' life, of which the mechanism is still elusive. Directevidenceindicatesthathistonemodificationplaysanimportantroleinneuropathic pain. Our recent findings revealed that histone deacetylase 5 (HDAC5) among these HDAC family members was differently expressed in spinal dorsal horn after partial sciatic nerve ligation surgery (pSNL), and plays a critical role in the development of neuropathic pain. We demonstrated that pSNL-induced mechanical allodynia and thermal hyperalgesia in mice were associated with increased mRNA and protein expressions of HDAC5 and SRY-related HMG-box 10 (SOX10) in the ipsilateral lumbar dorsal horn. Gene knockdown of spinal HDAC5 or SOX10 by siRNAs attenuated pSNL-induced nociceptive hypersensitivity, companies with the decrease of spinal neuronal sensitization markers, including phosphorylated-Erk (p-Erk), phosphorylated-GluN1(p-GluN1) (ser896) and c-Fos. Conversely, overexpression of spinal HDAC5 or SOX10 by lentiviruses induced pain-like behaviors and increased

the expression of these spinal neuronal sensitization markers in naïve mice. Additionally, different to its conventional deacetylation effect, overexpression of HDAC5 through a non-deacetylation regulation not just increased SOX10 expression but also induced hypersensitivity in naive mice, which were reversed by SOX10-siRNA. Chromatin immunoprecipitation assay further confirmed that a previously unidentified modulation of non-histone protein by HDACs that HDAC5 regulates SOX10 by binding to the promoter region of the Sox10 gene. In conclusion, this study for the first time demonstrates that HDAC5 regulates spinal neuronal sensitization in neuropathic pain by modulating SOX10 expression. Thus, interventions that reduce HDAC5/SOX10 expression may represent promising avenues in the treatment of neuropathic pain.

S47-0003

Epigenetic therapy as a new target in anti-lung cancer drug discovery

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Epigenetics is a kind of heritable change in gene expression and without DNA sequence alteration. Lung cancer is the most common cause of death all over the world, and non-small cell lung cancer (NSCLC) accounts for 78%. Genetic changes of driver genes have been identified in NSCLC, including KRAS, EGFR, ALK translocation, ROS1 translocation, BRAF and HER2 et al. Other than genomic changes, epigenetic alterations such as changes in DNA methylation, histone modifications, and chromatin organization (e.g., nucleosome remodeling) impact gene expression and cellular gene function, and play an important role in the onset and progression of cancers. This review expounds the role of epigenetic regulation in lung cancer, as well as the field of epigenetic clinical has a potential in cancer treatment.
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S48-0001

Exome sequencing of major depression disorder (MDD) patients with extreme phenotypes

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Background: Exome sequencing is an efficient strategy to selectively sequence the gene coding regions of the genome. In the past few years， this technology has been widely used to study the genetic basis of psychiatry diseases. However, only the strategy that used trios to find *de novo* mutation is currently accepted. Another promising strategy is to sequence individuals who have extreme phenotypes.

Methods: We sequenced the exome of major depression disorder patients with extreme phenotypes to increase the detection effectiveness. Twenty MDD patients were selected based on their clear family history and an earlier age of onset. The raw sequencing data were firstly filtered with common variants (MAF> 5%), and then the genes with four or more missense mutations present in the 20 exomes has been identified.

Result: Fifty-five genes were survival after the filtration procedures, some of which were previously identified in genome wide association studies (GWAS), including COMT and SYNE1. Interestingly, clustering analysis found that receptor-coding genes are enriched in our result. Many mutations that are potentially harmful to normal expression or protein function have been identified in genes coding AMPA and KA receptors (ionotropic glutamate receptor), and GRM6, GRM7 (metabotropic glutamate receptor). Another group of genes are functionally associated with 'regulation of actin cytoskeleton', including the MAPK, PI3K/mTOR, and AKT signaling.

Conclusion: Both the genetic factors and external stimulus are critical for the development of MDD, but only a few causal genes have been found in the past. The strategy we used to sequence MDD patients who exhibit extreme phenotypes seems to be effective to detect real pathogenic mutations since some GWAS-identified genes were replicated in this study. Moreover, the present findings suggest that disruption in ion channel function and synaptic plasticity may be important for the development of MDD.

S48-0002

Copy number variation based genome wide association study of Hirschsprung's disease using whole genome sequencing data

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Copy Number Variations (CNVs) are defined as DNA segments larger than 50 base-pairs for which copy number differences have been observed. These structural variations can alter gene dosage, disrupt coding sequences, or affect gene regulation. CNVs are known to underlie Mendelian traits, complex diseases and sporadic genomic disorders and without a doubt represent an important portion of missing heritability in human diseases. CNVs discovery is essential for uncovering genes/risk factors for a wide range of diseases, including Hirschsprung disease (HSCR). Incidentally, gross deletions encompassing RET or EDNRB lead to the discovery of these two main HSCR genes. Also, our previous CNV analysis of HSCR

patients identified the association of CNVs encompassing NRG3 with HSCR, therefore vindicating a role for CNVs in HSCR.

A comprehensive CNV analysis was performed on whole genome sequencing data (Pair-end 150bp, ~30x) generated from 443 HSCR patients and 493 matched controls. As CNV calling is still a challenge, we used four complementary tools: CNVnator, Delly, Lumpy and Seeksv. CNVs detected by at least three tools remained for further analysis. Moreover, to ensure calling accuracy we have developed a new program whereby the predicted breakpoints are carefully inspected. The overall burden test for rare CNVs (<1%) identified increased number of rare duplications per individual as well as increased proportion of subjects with rare small deletions in case. The association analysis found 9 deletions and 3 duplications with P-value less than 10-4. Genes encompassed by the significant genic CNVs include TRPC6, NXPE1, NBPF20, NBPF9, PDE4DIP and TSGA10. These results reinforce the association of CNVs in HSCR, especially for the small CNVs which were undetectable in previous array-based technologies. The newly discovered HSCR-associated CNVs will enrich the repository of HSCR candidate genes and may contribute to the discovery of cell therapy for HSCR.

S49-0001

The genetic diversity of Aspergillus flavus in China

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Aspergillus flavus is the major aflatoxin B producing fungi which is also pathogenic to humans and animals causing aspergillosis, and frequently discovered in temperate, subtropical, tropical and arid and high-temperature areas, though it is commonly regarded as saprobic and widely distributed in the environment. Each year in the world, huge amounts of corns, peanuts and cottonseeds are vulnerable to be contaminated by *A. flavus* and aflatoxin, which is even worse in China. There is much variance in morphological, genetic and toxin-producing characters among isolates of *A. flavus*, which made the species concept unclear. In this paper, we analyzed 88 isolates from different environments in the temperate, subtropical, tropical and Qinghai-Tibet plateau climate areas including 26 provinces of China, together with 15 type cultures and authentic isolates based on multi-locus sequence typing (MLST) and toxin-producing features, and drew the following conclusions:

1. No high toxin-producing species, namely *A. minisclerotigenes* and *A. parvisclerotigenus* were found in China, which can produce aflatoxin B, G and CPA.
2. The 88 isolates isolated from China were distributed in three populaions, among which isolates of *A. oryzae* constitute one of the populations.
3. Different populations are not correlated with geographic environments; toxin-producing feature is isolate-specific and not correlated to populations; all the isolates of *A. oryzae* do not produce aflatoxin B.

S49-0002

Communication of traditional knowledge to improve sustainable agriculture

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Most of the agricultural traditional techniques are straightforward and inexpensive. They can be used effectively in developing society or remote communities. These methods are not only easy to use but environment friendly which improve sustainable development. One of the main aspects of traditional knowledge is that the wisdom of the past locally applied and remained in small community. Farmers are usually skeptical about new technology due to the detrimental effects if things go wrong. However, traditional knowledge is more acceptable since it has been gathered in generations through trials and errors for many centuries. Furthermore, these techniques can be tailored to local conditions. We present some of unique folk knowledge that collected from local tribes and suggest that the professional body such as Food and Agriculture Organization (FAO) lunch an initiative to promote collection of traditional knowledge from diverse community, then the credibility of such folk knowledge must be examined rigorously. Communication of the approved experiments in innovative fashions such as hands-on workshops, social media or interactive platforms on the web will connect smallholder farmers to reliable knowledge. Considering the needs and concerns of society, collaboration of communication science and

agricultural expert can help to find a holistic measure for sustainable crop production and achieve much better down to earth results.

S50-0001

THE PEG ASSOCIATED SOLVENT SYSTEM (PEGASOS) METHOD ENABLES 3-DIMENSIONAL VISUALIZATION OF HARD TISSUE AND SOFT TISSUE

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Recently tissue-clearing approaches have become important alternatives to standard histology approaches. Tissue clearing technique enables visualization of opaque organ and tissue in 3-dimension by turning tissue transparent. However, all current tissue clearing methods are restricted by limited types of tissue that can be cleared with each individual protocol, which inevitably led to the presence of blind-spots within whole body or body parts imaging. Hard tissues including bones and teeth are still the most difficult organs to be cleared. In addition, endogenous fluorescence loss remains to be a major concern for solvent based clearing methods. Here, we developed a polyethylene glycol (PEG) Associated Solvent System (PEGASOS), which efficiently rendered nearly all types of tissue transparent and preserved endogenous fluorescence thereby making it possible to imaging both hard and soft tissue separately or together at single cell resolution. In our research, we collected various tissues from different types of transgenic mice and cleared them following sequential steps including decalcification, decolorization, delipidation, dehydration and final clearing in PEGASOS. We found PEGASOS efficiently clears hard tissue and provides superior florescence protection. It turns the whole adult mouse body transparent and we were able to image under two photon microscope an adult mouse head composed of bones, teeth, brain, muscles and other tissues with no blind areas. Superior hard tissue transparency enabled us to reconstruct intact mandible, teeth, femur or knee joint in 3-D. Improved transparency and fluorescence preservation enabled us to image intact mouse brain at sub-cellular resolution to trace individual neurons and axons over a long distance and to visualize dorsal root ganglions directly through vertebrae. Therefore, for the first time, we revealed the neural network and vasculature system distribution pattern in 3-dimension within the long bone marrow space.

S53-0001

Stuxnet modulate octopamine effect on sleep through a stx-polycomb-octB2R cascade

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Octopamine has an important regulatory role in sleep. Octopamine induces loss of amount sleep as well as homeostasis, thus was inferred to have a role in the stressful situations for insects, like escaping from a harmful habitat. It is conceivable that counteracting mechanisms were needed to prevent the harmfulness caused by decreased sleep. We found that an epigenetic regulator *stx* was inhibited by octopamine to tune down one of its receptors *octß2R* through a *stx*-Polycomb-*octß2R* cascade. *octß2R* mediated the octopamine function in both sleep and homeostasis. As a result, the sleep decreasing and loss of homeostasis effects of octopamine on sleep was compensated.

S54-0001

Magnoflorine with Hyaluronic Acid Gel Promotes Subchondral Bone Regeneration and Attenuates Cartilage Degeneration in early Spontaneous Osteoarthritis

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Introduction: Aim to address Chinese medicine magnoflorine combined with hyaluronic acid (HA) gel effectively promoting trabecular bone remodeling and attenuate cartilage degeneration in spontaneous osteoarthritis (OA).

Method: The MC3T3 cell (osteoblast) viability was detected by XTT. The cell proliferation was reflected by cell cycle assay. Bone formation was detected by Alizarin Red staining. The 5 month female Dunkin-Hartley guinea pig as a spontaneous OA model was made a standardized bone defection on the tibial medial side. Guinea pigs were once intra-bone injected 50 ng magnoflorine, HA gel, 50 ng magnoflorine + HA gel, and null, respectively. All collected limbs were performed micro-CT (uCT) scan and histological staining at 2 month post-surgery.

Results: MC3T3-E1 treated with 25 μg/ml magnoflorine, the viability, S phase and mineralization were significantly increased. For in vivo study, with 50 ng magnoflorine + HA gel treatment, the trabecular bone parameters, such as BV/TV, BMD, Tb.N, Conn.Dn were increased, and DA was decreased, which implied trabecular bone regeneration. Furthermore, Treatment also resulted a decrease in Mankin's scores, lower tidemark thickness, higher volume ratio hyaline cartilage (HC)/ calcified cartilage (CC) and fractal dimension (FD, roughness indicator of osteochondral conjunction), when compared to Defect and HA-gel groups. Furthermore, FD was positively associated with volume ratio of HC/CC and negatively associated with modified Mankin's scores. Finally, histological results in HA-gel + magnoflorine treatment showed the elevated cartilage matrix, chondrogenic signals and prechondrocytes.

Conclusion: We elucidated the potential benefit of magnoflorine combined with HA gel for trabecular bone and cartilage regeneration, and the relationship between SBP and cartilage on the early stage of OA. It also demonstrated the possibility to diagnose pathogenesis of early OA using uCT analysis for clinical assessment.

S54-0002

Burden of hospitalized pneumonia in Hong Kong: a population-based estimation from 2011-15

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Objective: To characterize the burden of hospitalized pneumonia in Hong Kong – an urban city with high population density, long life expectancy, and a rapidly ageing population – to inform targeted healthcare policies for pneumonia control in the era of global ageing.

Methods: Patients admitted to public hospitals with a diagnosis of pneumonia at discharge were identified based on the International Classification of Diseases-Ninth Revision-Clinical Modification codes (480-486 and 487.0). Incidence, inpatient case-fatality, all-cause fatality, 28-days readmission, hospital length of stay, and healthcare costs were assessed in seven age strata.
Results: We identified 323 992 patients (median age 80 years, 44.4% female) with hospitalized pneumonia (organism unspecified: 84.2%; bacterial pneumonia: 12.3%; viral pneumonia: 2.5%; others: 1.0%) over five years. Annual incidence was 955.1 per 100 000 population, with a 10.6% decrease from 2011 to 2015. Case-fatality, all-cause fatality and 28-days readmission risks were 13.8%, 21.6%, and 19.5%, respectively. Average hospital length of stay was 14.1 days with corresponding direct costs of $9348 per episode in the monetary value of US Dollars 2015. Individuals ≥65 years accounted for over 75% of pneumonia-related hospitalizations, 90% of deaths and the majority of healthcare costs.
Conclusions: Hospitalized pneumonia, especially in older adults, represents considerable health and economic burden in Hong Kong. Facing a global ageing population, it is important for health systems to develop multidimensional and cost-effective strategies, to prevent and manage pneumonia in older adults. Findings from this study provide a baseline estimate of the burden of pneumonia hospitalizations for further evaluation of targeted strategies for pneumonia control.

S54-0003

Identifying Frailty in Clinical Practices: A Two-step Pathway

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Frailty is a state of vulnerability to stressors in older adults and is associated with worse prognosis. Frailty is common and contributes to rising health care costs. Frailty screening and assessment is a fundamental issue in primary care for clinicians, researchers, and health care organizations. Early screening and timely tailored intervention may effectively prevent or delay the adverse outcomes in older adults. Frailty measures should be evaluated in line with the goal of the frailty tool in trying to understand the underpinning biology, to help with diagnosis and care planning, or to stratify risk for worse outcomes. However, the reliability of many frailty assessment tools has not yet been verified. Thus far, no study has demonstrated an optimal assessment tool for guiding clinical management, and the selection of an assessment tool still relies on specific clinical conditions. Currently, self-reported screening tools can identify frailty and predict the risk of adverse outcomes in older adults. Because they are easy to use and quickly provide information, self-reported frailty screening tools have significant implication in primary care setting and clinics. We reviewed the frailty screening instruments in older adults and proposed a two-step pathway for frailty identification, and to manage declines in intrinsic capacity as well as boost resilience. The first step (case-finding) is performed by non-specialist staff or participants themselves, using self-reported screening tools. The second step (assessment) is conducted by trained professionals using complex, time-consuming or equipment required performed frailty assessment tools or CGA, which will be followed by comprehensive care planning, including personalized intervention for frailty to delay decline in intrinsic capacity and boost resilience.

S55-0001

Transcorneal Electrical Stimulation Inhibits Retinal Microglial Activation and Enhances Retinal Ganglion Cell Survival After Acute Ocular Hypertensive Injury

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Purpose: To investigate the effect of transcorneal electrical stimulation (TcES) on retinal ganglion cell (RGC) function and survival after acute ocular hypertension-related retinal injury in gerbil eyes.

Methods: Gerbil eyes were subjected to acute ocular hypertensive injury (80 mm Hg for 60 minutes). In the treatment group, TcES was applied to the surgical eye immediately and then twice weekly for a total of 1 month. In the control group, sham TcES was given to the surgical eye at the same time points. Retinal function was assessed and compared between groups using flash electroretinography. For histological analysis, the number of RGC and microglial cells were counted by immunofluorescence staining after the gerbils were sacrificed on day 7 and day 28. Real-time polymerase chain reaction and western blot analysis were conducted to compare expression of interleukin (IL)-10, IL-6, COX-2, tumor necrosis factor (TNF)-α, and NF-κB phosphorylation among groups.

Results: TcES-treated eyes had significantly higher RGC survival at 1 month compared to controls. This was associated with RGC function. Furthermore, TcES-treated eyes were shown to have increased IL-10 expression, with a corresponding reduction in IL-6 and COX-2 expression as well as reduction in NF-κB phosphorylation. This was associated with a suppression in microglial cell activation in TcES-treated eyes.

Conclusions: Early treatment with TcES in gerbils protected the RGC from secondary damage and preserved retinal function in acute ocular hypertensive injury through modulation of the microglial-cell activated local inflammatory response.

S55-0002

Topological Analysis of Protein Folding of LRRK2 Mutants in Parkinson's Disease

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Mutations in the Leucine-rich repeat kinase 2 (LRRK2) gene, which codes for proteins that play significant roles in maintaining normal brain function, are linked to an increased risk of Parkinson's Disease (PD). Although it is still unclear how exactly point mutations happened on the molecular level during the early development of PD lead to various clinical cases, researchers have used animal models, such as zebrafish and mice, to understand chemical changes in the brain. Recently, as over 1000 proteins have been classified with specific mathematical knot types, I focus on possible connections between topological changes in mutated proteins and the pathogenicity of mutations. I use computer software to characterize 13 point mutations and study the topology of sections of proteins. I studied 13 single nucleotide variants in LRRK2 from NCBI variation viewer, predicted the mutated proteins using Phyre2, extracted their protein backbones from PDB files, and modeled the 3D structures of backbones in KnotPlot where I generate topological parameters and identify the knot type of each mutated protein and

its wild-type. From there, I can find the knot type change of a protein backbone due to a point mutation. I hypothesized that mutations would change the topology of the protein backbone. The results prove that LRRK2 mutations change the topological and geometrical structure of the protein backbone.

S55-0003

Targeting PTPσ ameliorates morphological and behavioral outcomes after experimental ICH in mice

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One reason for limited recovery from intracerebral hemorrhage (ICH) may be the development of a glial scar at the border between normal and damage tissue. The glial scar, which contains reactive astrocytes and associated extracellular matrix (ECM) proteins such as CSPGs, not only suppresses axon regeneration, but also results in demyelination due to oligodendrocytes death in both the immediate vicinity of the injury site, thereby interfering with long-term anatomical and functional recovery. CSPG inhibition of axonal regeneration and oligodendrocyte outgrowth and myelination is partially mediated by the protein tyrosine phosphatase sigma (PTPσ) receptor. Intracellular sigma peptide (ISP), a newly developed membrane permeable peptide binds to PTPσ and relieves CSPG-mediated inhibition has been used to promote regeneration and functional recovery after spinal cord injury and ischemic heart attack. We hypothesize that treatment with ISP would lead to functional recovery through enhancing axon regeneration and remyelination after intracerebral hemorrhage. Experimental ICH model in male mice was induced by intrastriatal injection of Collagenase IV. The functional neurological recovery were evaluated weekly until 8 weeks post-ICH using rotarod test and Cylinder test following daily ISP subcutaneous injection over 8 weeks. Perilesional axonal regeneration and morphological change of the corticospinal tract at cervical spinal cord was assessed by histological staining, light and electron microscopy, as well as immune blot. Functional recovery and increased perilesional axonal sprouting as well as recovery of injured ipsilesional CST were observed in ICH mice with ISP treatment. These results suggest that modulation of PTPσ by ISP represents a potential therapeutic strategy for hemorrhagic stroke.

S55-0004

Neuro-protective Effect of Rsveratrol on Ischemia/Reperfusion-induced Retinal Injury in mice

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Retinal ischemia/reperfusion (RIR) injury is a common pathological cause of visual impairment. Resveratrol is a natural phenolic phytoalexin produced by plants. The benefits of resveratrol in neuroprotection have been found in various neurodegenerative diseases; however, limited evidence was present for RIR-induced injuries. In this study, we established the RIR injury model by increasing

intraocular pressure to 95mmHg for 90 minutes in the right eye of male C57BL/6J mice. Afterward, the mice were divided into the resveratrol treatment (20mg/kg) and the sham treatment group. The treatment was done by intraperitoneal injection and was initiated on the day of IOP elevation, and repeated daily for 1 week and 4 weeks respectively. Resveratrol treatment for 1 week and 4 weeks significantly reduced the percentage of RGC loss from 38.43±10.63(%) to 26.45±6.77(%) and from 40.27±11.19(%) to 25.17±7.35(%) ($p<0.05$) respectively. The amplitude of a wave and b-wave from electroretinogram was recorded for the evaluation of the retinal function. Resveratrol treatment for 1 week and 4 weeks reduced the percentage of a-wave amplitude decrease from 41.85% to 21.99% and from 52.8% to 33.00%, though not significantly. Resveratrol treatment for 1 week and 4 weeks reduced the percentage of b-wave amplitude decrease from 47.38% to 21.64% ($p<0.05$) and from 45.60% to 24.00% ($p>0.05$). One week after RIR injury, no difference in SIRT1 and Cox-2 expression level was found between two groups. Four weeks after RIR injury, SIRT1 expression level in resveratrol treatment group was significantly upregulated ($p<0.05$) and Cox-2 expression level in resveratrol treatment group was significantly downregulated ($p<0.001$). In conclusion, resveratrol was effective in protecting RGC from degeneration induced by RIR injury in mice. The underlying mechanism might be the upregulation of SIRT1 which downregulates the expression of COX-2.

S56-0001

mRNA therapeutics: A different vision

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In vitro transcribed (IVT) mRNA is forming a new basis of drug delivery as it can be synthetically engineered to express the desired protein in lesser time and be more specific than the other 2 types of antigens. Using DNA (coding for proto-oncogenes) for immunization poses a risk for host cell transformation into a malignant one and hence proto-oncogenes would express for a longer time. Therefore, mRNA was considered for cancer immunotherapy as it does not integrate into the host cell's genome, so no question of insertional mutagenesis arises. Our main aim is to develop a stable mRNA system working both *in vitro* and *in vivo* and then use this system to develop mRNA vaccines. The stability of IVT mRNA can be increased by changing the 3' and 5' UTRs, adding a 5'cap and 3' poly-A tail which could even increase their half-life to a few days. For creating a stable mRNA working system in vitro, EGFP-2A gene was cloned along with the AdsA (*Staphylococcus aureus* gene important for infection) in a vector backbone with TMV 3'UTR and 5'UTR. The mRNA created in vitro was with modified uridine and cap 1 structure and transfected to the mammalian cell lines. This transfection was further enhanced by using liposome created in the lab (using some cationic and helper lipids in different ratios) by film rehydration method. The expression of luciferase mRNA was then detected in mice by live imaging indicating that the mRNA system is stable *in vivo*. Further, to direct the mRNA encoded antigens to MHC1 and MHC2 pathways they were tagged with a htPA secretion tag and MHC1 targeting signal along with a 3XFLAG tag. We tried various mode of injections with AdsA mRNA to have a profile of ELISPOT with differently exposed mRNA (*in vivo* in mice). Now we are focussing on some neo antigens for tumor to be used as a vaccine in the form of mRNA.

S58-0001

Diterpene ginkgolides protect rat brain from I/R damage

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Diterpene ginkgolides meglumine injection (DGMI) has been used for cerebral ischemic stroke treatment in China. Ginkgolides A, B and C are the main components of DGMI. Acute cerebral ischemic injury was induced in rats by occlusion of the middle cerebral artery (MCA) for 1.5 h followed by 24 h reperfusion *in vivo*. DGMI improved neurological deficit, attenuated cerebral edema and reduced infarct volume in I/R injury rats?. Neurological deficit scores were assessed after 24 h of reperfusion to determine the protective effects of DGMI. The DGMI 1, 3 and 10 mg/kg groups exhibited dose-dependent decreased neurological deficit scores. The recorded typical movement route of rats also showed high neurological deficit scores in the I/R group, while low neurological deficit scores were observed in the DGMI treatment groups. DGMI also significantly reduced brain infarct volume, inhibited brain cell apoptosis, and induced protein kinase B (Akt) phosphorylation, which prompted the nuclear translocation of nuclear factor-erythroid 2-related factor 2 (Nrf2) and phosphorylation of the survival regulatory protein cyclic AMP-responsive element binding protein (CREB). Nrf2 activation led to expression of the downstream protein heme oxygenase-1 (HO-1). These observations suggest ginkgolides as novel extrinsic regulators activating both Akt/Nrf2 and Akt/CREB signaling pathways, protecting against cerebral ischemia/reperfusion (I/R) damage in a rat model.

S58-0002

Esculin alleviates LPS-induced acute lung injury in mice involving down-regulation of TLR4/MyD88 pathway

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Acute lung injury (ALI) is a clinical symptom associated with high morbidity and mortality in diseases such as shock, sepsis, and cerebral ischemia and reperfusion. The inflammatory response plays a key role in the development of ALI. Lipopolysaccharide (LPS), a classic anti-inflammatory agent, mediates a typical inflammatory response in pulmonary tubular epithelial cells by activating the TLR4 signaling pathway. This study was to investigateeffect ofesculin (ES) on LPS-induced acute lung injury and its mechanism of action in mice. The mice were divided into normal group, model group, ES 25, 50, 100 mg/kg treatment groups. After seven days of pre-administration, LPS 5mg/kg was injected intraperitoneally and lung tissues were taken after 6 hrs. The expression of inflammatory factors and related inflammatory protein in lung tissues were measured by ELISA and western blotting. The results showed that ES could reduce lung injury and inhibit the level of pro-inflammatory cytokines including IL-1β, IL-6, TNFα, MCP-1 and ICAM-1 induced by LPS. In addition, ES also down-regulated LPS-inducedTLR4/MyD88 inflammatory pathway activationinduced by LPS. This suggested that ES could improve LPS-induced ALI and inflammation in mice,and its anti-inflammatory effect may be related to inhibition of expression of TLR4 / MyD88 inflammatory pathway related proteins.

S58-0003

Molecular Docking Study on the Vasomotor Material Basis of Xiao-Xu-Ming Decoction

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Xiao-Xu-Ming decoction (XXMD) is a traditional Chinese herbal medicine used for the treatment of stroke. The balance shifting from vasodilatation to vasoconstriction is a key initiator of stroke. XXMD was reported to exert a positive effect on cerebral circulation in animal models of stroke, but the mechanisms of its vascular regulatory function and the interactions among multi-components of XXMD remain to be elucidated. The study utilized a molecular docking approach based on network pharmacology to uncover the potential synergistic effects of the components in different herbs of XXMD on vasoconstriction and vasodilatation regulation. After constructing and analyzing the database containing 963 compounds of XXMD, the interactions between eight receptor linked to vasoconstriction (5-HT1AR, 5-HT1BR, AT1R, β2-AR, UTR, ETA, AGTR1, V1AR) and six receptor linked to vasodilatation （ADORA2A, CALCRL, NPRA, NPRB, APJ, ETB）and various herbal compounds were assessed using molecular docking. By constructing and analyzing the chemical composition-target networks for XXMD, *Radix Glycyrrhizae*, *Radix Ginseng* and *Radix Paeoniae Alba* were found to have the large quantity of high docking scores compounds. Furthermore, thirty-eight compounds in XXMD such as Gingerenone C, isoliquiritin apioside and 1,2,3,6-Tetra-O-gafloyl-β-D-glucose were found to target multiple receptor closely related to vasoconstriction and vasodilatation. Therefore, the network interactions between varies of chemical components in XXMD and the vasoconstriction and vasodilatation targets may result in the comprehensive regulation of cerebrovascular function of XXMD, and thus constitute the vasomotor material basis of XXMD. Our results revealed the features and contributions of various components and herbs in this formula to its vasoconstriction and vasodilatation regulatory effects, providing scientific evidences for the multi-component and multi-target mechanisms of the drug.

S58-0004

The research of active constitutes of 50 Ganoderma triterpenoids on neuroprotection

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To investigate the protective effect of Ganoderma triterpenoids on the neural cells, understand the role of triterpenoids of Ganoderma in treating diseases, also find out the distribution of activity of various constitutes, we established six damage model of different mechanisms on both SH-SY5Y and PC12 cells, including corticosterone, KCl, L-glutamic acid sodium salt (MSG), sodium nitroprusside (SNP), Na2S2O3 and H2O2. MTT assay was used to detect the cell proliferation. The cultured cells were randomized into three groups: normal control group, injury agent treatment group and 50 Ganoderma triterpenoids treatment group (10 μmol/L). After 24 h, we found that Ganoderma triterpenoids had

obvious protective effects on six damage models especially on KCl and MSG injuries. The number of active constitutes on the six damages models as previously stated on SH-SY5Y cells was 3, 42, 24, 31, 13, 45 and on PC12 cells was 31, 30, 37, 4, 7, 22. The cell damage inhibition rate of the plurality of active constitutes is more than 80%. Combining two kinds of neural cells, Ganoderic Acid D, Ganoderic Acid B, Ganoderma acid C2, Ganoderenic Acid C, Lucidenic acid LM1, Ganoderenic Acid B, Lucidenic Acid B, Ganoderic Acid TR showed significant protection on at least 8 different damage models. Meanwhile, these 8 constitutes had no significant effect on the proliferation of normal neural cells, showing further research implications. Also, we found that several triterpenoids caused further cell damage, especially the Ganoderic Acid TN and Methyllucidone that we suggested were more suitable for treating tumors than neuroprotection. This study firstly explored the active constitutes of Ganoderma triterpenoids, proved their neuroprotective effect, and will provide a material basis and theoretical basis for the application of Ganoderma lucidum in mental diseases.

S58-0005

Discovery and in vitro efficacy evaluation of multitarget active compounds against influenza A virus

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Influenza A virus (IAV) can cause the global pandemics and epidemics, which remain unpredictable threats to public health. In recent years, the novel therapeutics against IAV are required urgently by the high mutation rate of IAV and drug resistance of existing drugs. In this investigation, 15 targets selected from the viral component-host factor interaction network were successfully constructed as a multitarget anti-IAV prediction model using Naïve Bayesian (NB), Recursive Partitioning (RP) and CDOCKER methods, and the model's prediction accuracy and applicability were verified. Then we collected the constituents of Compound Yizhihao (CYZH), consists of Radix isatidis, Folium isatidis and Artemisia rupestris, which is effective traditional medicines for influenza treatment. After being predicted by multitarget prediction model, 28 compounds with multitarget activities were selected for in vitro efficacy evaluation. In the neuraminidase (NA) inhibition experiment, 9 compounds showed inhibitory activity against the NA. Hemagglutinin (HA) inhibition test results showed that all compounds had no obvious activity on HA. In the experiment of the effect on Tumor necrosis factor alpha (TNF-α) content, 20 of the 28 compounds showed significant differences. The results of cellur cytopathic effect (CPE) reduction assay caused by the common strains showed that acacetin, indirubin, tryptanthrin, quercetin, luteolin, emodin and apigenin had good activity; resistant strains-induced CPE reduction assay showed that dinatin, quercetin, luteolin and apigenin had good efficacy. Finally, with the aid of constituent-target-pathway network, the effective constituents and their network mechanism of action in CYZH were revealed. Taken together, these findings will provide theoretical tool for discovery of new anti-influenza compounds and important approach for traditional Chinese medicines study in IAV treatment.

S58-0006

Mechanism study and uric acid reduce effects of allantoin on potassium oxonate induced hyperuricemia in mice

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The purpose of this study was to investigate the effects of allantoin in hyperuricemia induced by potassium oxonate in KM mice. Hyperuricemia mice model was established by confirming elevated levels of serum uric acid and creatinine after intraperitoneal injection of potassium oxonate. Mice were then treated with daily oral doses of allantoin for 14 days in parallel with mice treated with allopurinol and benzbromarone as positive controls. The levels of uric acid were monitored at 2 days intervals. The levels of other biochemical indexes were determined by biochemical analyzer and nephritic and hepatic symptoms observed by hematoxylin and eosin (H&E). Western blot and qRT-PCR analysis of renal tissue was also employed. Allantoin treatment caused a significant reduction in the levels of serum uric acid and reduced renal histopathological changes and pathological effects. Allantoin treatment also significantly increased the mRNA expression of URAT1 and OAT1 in renal tissues of hyperuricemia mice. Meanwhile, allantoin can increase the mRNA expression of GLUT9 in ileum. In conclusion, the results suggest that allantoin reduce serum uric acid in potassium oxonate-induced hyperuricemia in KM mice through increment of mRNA expression of URAT1 and OAT1 in renal and GLUT9 in ileum.

S58-0007

Puerarin Inhibits PDGF-BB-Induced Vascular Smooth Muscle Cell Proliferation and Migration via PDGFR/ERK Signaling

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Abnormal proliferation and migration of vascular smooth muscle cells (VSMCs) contributes to the pathogenesis of cardiovascular diseases, including atherosclerosis and restenosis. Puerarin, an active ingredient from the traditional Chinese herbal medicine pueraria lobata., has been found to exhibit many bioactive functions such as anti-inflammatory, vasorelaxation and anti-oxidant. The aim of the present study was to explore the anti-proliferative and anti-migration effects of puerarin on VSMCs and the possible mechanisms. VSMCs were cultured and pretreated with different concentrations of puerarin (0, 3, 1, 3, 10, 30, 100 μM) before stimulated by platelet-derived growth factor (PDGF)-BB (25 ng/ml). VSMC proliferation and migration were measured by crystal violet staining, wound-healing and boyden chamber assays, respectively. Cell cycle was analyzed by flow cytometry. Expression of adhesion molecules, cell cycle regulatory proteins, the phosphorylated levels of PDGF receptor β (PDGF-Rβ), extra-cellular signal regulated kinase (ERK), Akt, p38, JNK and PLC-γ1 were tested by western blot. The results indicated that pretreatment with puerarin dose-dependently inhibited PDGF-BB stimulated VSMC proliferation and migration, which were associated with a cell-cycle arrest at G0/G1 phase, a reduction in the adhesion molecule expression. Furthermore, the increase in PDGF-Rβ, ERK1/2, Akt, p38, and JNK phosphorylation induced by PDGF-BB were suppressed by puerarin. These findings indicate that puerarin inhibits PDGF-BB induced proliferation and migration of VSMCs by suppressing PDGFR/ERK signaling pathways. In conclusion, the present study suggests that puerarin may be beneficial as an anti-proliferative agent for the treatment of vascular diseases.

S58-0008

Xiaoyao San and its core herb-pairs Radix Bupleuri-Radix Paeoniae Alba exert antidepressant effect by against liver injury induced by chronic unpredictable mild stress model in rat

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Xiaoyao San (XYS) is a famous classic TCM formula for treatment of liver stagnancy and depression.TCM theory and modern research show that there is a connection between depression and liver injury, liver injury was induced in CUMS model. However, The mechanism is unclear. Therefore, we investigated whether CUMS-induced liver injury is associated with inflammatory response and oxidative stress in rat liver, In addition, the changes of metabolites were analyzed by metabolomics, we make full advantage of the complementarity between NMR and UPLC-MS, the metabolites of liver tissues were analyzed using UPLC-MS and NMR. The results indicated that both treatments significantly regulated depression-like behavior in CUMS rats and decreased the levels of ALT and AST compared to CUMS group, which indicated that both treatments has obvious antidepressant effect and can significantly improve CUMS-induced liver injury. Compared with the CUMS group, the two treatments can significantly increase the levels of SOD, CAT and GSH-Px in the liver of rats, indicating that the two treatments can enhance the antioxidant capacity in liver. The levels of apoptosis-associated Bax and Bcl-2 protein in liver tissue were significantly lower than those in CUMS group, suggesting that both treatments can inhibit liver cell apoptosis induced in CUMS by activating antioxidant pathways. Metabolomics results indicate that XYS regulated phospholipids and bile acid metabolism against liver injury induced by CUMS in rat. This study attempts to explain the potential antidepressant mechanism of XYS from the link between liver injury and depression, it also clarifies the core position of Radix Bupleuri-Radix Paeoniae Alba herb-pairs in XYS.

S58-0009

Toxicity and Efficacy of Petroleum Ether Fraction of Bupleuri Radix on Normal Rats with Chronic Unpredictable Mild Stress

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In our previous work, the petroleum ether fraction of Bupleuri Radix (PBR) have a therapeutic effect and a comparatively mild adverse effect in Chronic Unpredictable Mild Stress rats (with a depression pattern). Considering the report of "Rob 'yin' of liver by bupleurum". The present study apply Liver metabonomics to decipher the differential effective and toxic mechanism of Bupleuri Radix in CUMS and normal rats using the UHPLC-Q Exactive Orbitrap-MS technique. In this study, 136 male Sprague-Dawley rats were randomly divided into 17 groups of 8 rats: [K] healthy rats, [Z1] ~ [Z7] healthy rats given PBR (D1 ~ D7), [CM] CUMS rats, [CY] CUMS rats given venlafaxine hydrochloride，[C1] ~ [C7] CUMS rats given PBR (D1 ~ D7). Liver were collected for LC-MS metabonomics analysis on days 21. Our results demonstrated that the PBR could regulat amino acid metabolism, energy metabolism, sphingolipid metabolism and β oxidation of fatty acid based on liver metabolic profiles to produce an antidepressant effect in CUMS rats. And in healthy rats six metabolic pathways were significantly

perturbed by PBR, including amino acid metabolism, energy metabolism, sphingolipid metabolism and β oxidation of fatty acid, glycerophospholipids metabolism and bile acid metabolism. The results might be responsible for the different effect of PBR on the liver under different body states, reminding us the importance of syndrome differentiation of TCM. The differential effective and toxic mechanisms of PBR in CUMS and healthy rats support the traditional Chinese theory of "You Gu WuYun" recorded first in Su Wen, a classical Chinese medical treatise. Futhermore, it provides a new reference for assessing the more rational and safer application of clinical drugs in the future.

S58-0010

Deciphering metabonomic biomarker-protein targets interactions in depression by network pharmacology

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Depression is one of the prevalent and prominent complex psychiatric diseases and the number of depressed patients has been on the rise globally during the recent decades. 36 metabonomic biomarkers associated with depression were identified via proton nuclear magnetic resonance (1H-NMR), gas chromatography-mass spectrometer (GC-MS), liquid chromatography-mass spectrometry (LC-MS) coupled with multivariate data analysis in our previous work. To further explore the roles of metabonomic biomarkers in the pathogenesis of depression, the metabonomic biomarker-enzyme network was constructed. The key metabonomic biomarkers and enzymes were screened out by network analysis. Stearic acid, phytosphingosine, glycine, glutamine and phospholipids were the most important metabonomic biomarkers. Most key enzymes are hydrolase, transferase and acyltransferase. Nine proteins (*TP53*, *IL1B*, *TNF*, *PTEN*, *HLA-DRB1*, *MTOR*, *HRAS*, *INS* and *PIK3CA*) were extensively involved in nervous system, immune response and endocrine, and might be drug targets for depression. A docking score-weighted polypharmacological index was introduced to evaluate the importance of target-related pathways. PI3K-Akt signaling pathway, mTOR signaling pathway and five other pathways had close correlation with the pathogenesis of depression and could deserve further research. Application of these biomarkers in clinical practice may help to optimize the diagnosis of depression.

S58-0011

Atractylenolide III protects PC12 cells from corticosterone-induced cytotoxicity via regulating mitochondrial apoptotic pathway and MAPKs/NF-κB inflammatory pathway

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Atractylenolide III (ATL-III), a sesquiterpene compound isolated from *Rhizoma Atractylodis Macrocephalae*, has revealed a number of pharmacological properties including anti-inflammatory, anti-cancer activity, and neuroprotective effect. Recent studies demonstrated that ATL-III can significantly ameliorate learning and memory impairment induced by chronic homocysteine administration in rats and

prevent the neuronal apoptosis caused by glutamate, indicating an anti-depression effect of ATL-III. The purpose of the study is to evaluate the anti-depression activity of ATL-III on corticosterone injured PC12 cells and further explored potential mechanisms. Our results demonstrate that ATL-III protect PC12 cells in vitro, likely through the blockage of [Ca2+]i overloading, the mitochondrial dysfunction, consequent increase of the Bax/Bcl-2 ratio, release of cyt C and caspase-3 activation. Furthermore, the protective effects of ATL-III might have been exerted by suppressing the phosphorylation of JNK, p38, and ERK1/2, leading to suppressing of the activation of NF-κB signaling pathway and thereby blocking the release of proinlammatory cytokine TNF-α, which finally induced the blockage of the inflammation cascade. The findings clarify the molecular mechanism that ATL-III protected the PC12 cells against corticosterone-induced injury for the first time. Our results provide the evidence that ATL-III may act as a therapeutic agent in the treatment of depression and that the anti-depression effect of ATL-III is associated with immunomodulation.

S58-0012

Bioinformatic analysis reveals key genes and pathways in aging brain of senescence-accelerated mouse P8 (SAMP8)

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Aging is a complex process accompanied with the decline of the different physiological functions. Numerous differentially expressed genes (DEGs) have been found in the aging brain of senescence-accelerated mouse P8 (SAMP8), however, it was challenging to screen out the crucial ones.

This study aimed to explore the crucial genes and pathways in aging brain of SAMP8 mice, which would be beneficial for understanding the pathogenesis of brain aging. Firstly, 430 genes that are differentially expressed in SAMP8 mice versus SAMR1 mice were obtained from 9 gene expression studies, and gene-gene network was constructed. Clustering analysis and topological analysis were used to single out the hub genes from this network. Secondly, pathway enrichment analysis was utilized to identify the key pathways from the 430 DEGs, and the DEGs in key pathways were considered as functional genes. Thirdly, the inner-network between hub genes and functional genes was constructed, and the key genes were predicted. Parts of the key genes were experimentally verified by quantitative real-time PCR (qRT-PCR), and the associated transcription factors (TFs) were predicted. Our results revealed that 12 crucial genes might affect brain aging, including Trp53, Bcl2, Tnf, Casp9, Fos, Il6, Ptgs2, Il1b, Bdnf, Cdkn1a, Pik3c3, Rps6ka1, among which Casp9, Fos, Ptgs2, Cdkn1a, Pik3c3, and Rps6ka1 had been verified by qRT-PCR in 10-moth-old SAMP8 mice. Five functional groups including mitogen-activated protein kinase (MAPK) signaling pathway, neurotrophin signaling pathway, Hepatitis B, Alzheimer's disease and Oxytocin signaling pathway were significantly changed during aging process in SAMP8 mice. Two key transcription factors of c-Fos and C/EBPbeta were predicted by constructing a TF-target gene network. Our results indicate that these putative genes and pathways are closely related to brain senescence, which would gain new insight into the pathogenesis of brain aging.

S58-0013

Quality comparison study between absolute growth years' natural and cultivated Astragali Radix based on anti-heart failure efficacy

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The commercial specification grade of root kind of medicinal materials can not only reflect the quality, but also reflect the growth years of the medicinal materials. However, the growth years of Astragali Radix (AR) can not be reflected accurately by the standard. In this study, absolute growth years of natural Astragali Radix (NAR) was obtained by identifying the growth rings of the root.through the methods of paraffin-cut section and bare-handed section. The contents of flavonoids and saponins of NAR from different growth years' samples were determined by HPLC-UV-ELSD. Then, the intervention effects of NAR(4-year-old) and cultivated Astragali Radix (CAR) were compared in rat model with heart failure induced by doxorubicin (DOX). The results showed that the numbers of growth ring number excepting hollow part is consistent with the actual growth period of AR, and the number of growth rings gradually decreased from the upper to lower. The results of HPLC-UV-ELSD determination indicated that the saponins content of 3-year-old AR was the highest while the flavonoids content of the 4-year-old reached the maximum. Pharmacodynamics results revealed that the anti-heart failure effect of rats were improved with both WAR and CAR. However, compared with the model group, the intervention effects of the NAR on cardiac function parameters (EF, FS, and LVIDs), biochemical markers (CK) and serum BNP content were superior to the CAR, and showed significant differences. The results of this study provided a scientific basis for establishment of the specification grade and the clinical rational drugs use of AR.

S58-0014

Effects of Capsaicin on Migration Suppression of Hepatocellular Carcinoma Cell assessed by metabonomics and network analysis

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Capsaicin is a kind of alkaloid found in pepper cortex, which has extensive pharmacological activities. Capsaicin could be used as a potential drug for cancer prevention and treatment, and it has wide application prospect. However, the effects and mechanism of capsaicin for hepatocellular carcinoma has not been deeply studied so far. The aim of this study was to investigate the anti-cancer effects and mechanisms of capsaicin in SMMC-7721 cells by detecting the effects of proliferation, migration, adherence and invasion, and 1H-NMR based metabolomics approach. The results showed that capsaicin remarkably suppressed cell proliferation, migration, and invasion and hampered cell-matrix adhesion in human hepatocellular carcinoma SMMC-7721 cells. Metabolomics approach revealed that 17 differential metabolites regulated by capsaicin were identified inside and outside of the cells after

treatment of capsaicin. The metabolic pathway analysis suggested that Glutathione metabolism, Pyruvate metabolism, Phenylalanine metabolism, Glycine, serine and threonine metabolism, Glycerophospholipid metabolism are involved in the anti-cancer effects of capsaicin. We further reconstructed a metabolic network through MetScape based on the metabolites in five metabolic pathways that regulated by capsaicin. The constructed compound-target-metabolite network revealed the associations between the capsaicin and hepatocellular carcinoma. This study revealed that metabolomic integrating with network analysis can facilitate understanding the anti-hepatocellular carcinoma mechanisms of capsaicin.

S58-0015

Role of Saccharum Granorum as a "principal drug" in a traditional Chinese Medicine formula against chronic atrophic gastritis rats

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Saccharum Granorum (YiTang), a nutrient-rich food, has been used as traditional Chinese medicine (TCM) for thousands of years. However, the mechanism responsible for its beneficial efficacy is poorly understood, especially for its roles involved in the TCM formulas. In the present work, a classical TCM formulas, Huangqi Jianzhong Tang (HQJZ), was selected to interpret the efficacy and "principal drug" role of YiTang against chronic atrophic gastritis (CAG) rats using a serum metabonomic approach. 12 candidate metabolites were identified to characterize the difference between CAG and normal rats, which were involved into four disturbed metabolic pathways including glucose metabolism, amino acid metabolism, fatty acid metabolism and choline metabolism. The further PLS-RA revealed that five metabolites including choline, acetate, alanine, a-glucose and β-glucose were considered as potential biomarkers related to CAG. YiTang could exert the synergistic effect with HQJZ without YiTang, where the whole formula obtains a best beneficial treatment against metabolic disturbance induced by CAG. The metabolic improvement of YiTang and HQJZ were affected on the dysfunction of energy metabolism, gastric emptying and the changes of gut microbiome, which were the important pathomechanism induce by CAG. These findings suggested that YiTang played an indispensable role in the HQJZ formula against CAG, which may provide an approach to understand the fundamental idea behind formula construction.

S58-0016

Angelicae Sinensis Radix exert the anti-depression effect via modulating the blood system

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Angelicae Sinensis Radix (AS), a famous TCM herb wildly used in Asia for thousands of years, has been confirmed with anti-depression effect in recent years. Related studies suggested an association between depression and anemia, and that depression could be ameliorated via modulating the blood system. Traditionally used for replenishing the blood and promoting circulation, AS is speculated to exert the anti-depression effect via modulating the blood system. In the current study, CUMS combined with

metabonomics were performed to evaluated the anti-depression effect of AS and explored potential mechanisms. Our result demonstrated that AS significantly improve the depressive symptom induced by CUMS on SPT, OFT and FST. The analysis of blood routine examination suggested that AS could reverse the CUMS-induced haematological anomalies of RDW, MCV, RBC, PLT and MONO%. The analysis of blood gas showed that AS could regulate the CUMS-induced hypoxia. The result of metabonomics demonstrated that the AS group significantly separated from the CUMS group. Additionally, 25 potential biomarkers in depression could been regulated by the administration of AS. 8 biomarkers included valine, glucose, glycine, lactate, proline, citrate, sphingosine, and alanine could been regulated by AS in anemia reported in previous literatures. The shared metabolites regulated by AS both in depression and anemia were regarded as the anti-depression target that AS exerted via modulating the blood system.

S58-0017

Mutant IDH1/2 and Targeted Cancer Therapy

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Isocitrate dehydrogenase 1 and 2 (IDH1/2) are homodimeric enzymes that catalyze the conversion of isocitrate to α-ketoglutarate (α-KG) accompanied with the production of NADPH in TCA cycle. However, mutations in the genes encoding for these enzymes occur in various types of malignancies. Cancer-associated IDH1/2 mutations confer a new ability of these enzymes to catalyse the NADPH-dependent reduction of α-ketoglutarate to R-2-hydroxyglutarate (2HG). 2-HG has been proved to be an oncometabolite capable of promoting tumor development through competitively inhibiting the α-KG-dependent dioxygenases involved in histone and DNA demethylation, thereby impairing normal cellular differentiation and antitumor T cell immunity. Thus, a variety of approaches to inhibit the generation of 2HG have been explored. Recently, an increasing number of mIDH1/2 inhibitors have been developed to treat IDH1/2 mutated tumors. For example, enasidenib, a mutant IDH2 inhibitor, was discovered in 2008 and approved in 2017 for AML with IDH2 mutation. In addition, attributing to the fact that glutamine is a primary carbon source for 2-HG production in mutant IDH tumors, targeting glutaminase has been confirmed to be an option that selectively suppresses the growth of AML cells with IDH mutations. Besides, IDH mutations sensitize tumor cells to chemotherapeutics, proposing a distinct therapeutic strategy based on IDH mutations. In conclusion, these results provide new strategies for targeted cancer therapy based on the detection of IDH mutations.

S58-0018

A C-28 Oxidase, CYP716A249, is a key role in the biosynthesis of the Polygala saponins

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Radix Polygalae has been used in traditional Chinese medicine for its efficacy of tranquilization, glaangal, eliminating sputum, and detumescence for centuries. Triterpenoid saponins are the major contributors to

modern pharmacological functions, such as the traditional efficacy of glaangal, anti-aging, and brain protection. However, the structure and biosynthesis pathway of triterpenoid saponins are complex, the amount in plants is low, and the extraction cost is high. Therefore, we intends to synthesize polygala saponins by means of heterologous biosynthesis. In previous studies, it was found that *CYP716A249* may catalyze the synthesis of oleanolic acid from β-amyrin, which is a precursor of polygala saponins. Hence, the synthetic pathways of β-amyrin were constructed in *Saccharomyces Cerevisiae* by introducing β-amyrin synthase in this study. In addition, *ERG20* genes were overexpressed to increase the precursor supply for improving β-amyrin production. Then, synthetic pathway of oleanolic acid was constructed in *Saccharomyces Cerevisiae* to confirm the function of CYP450s involved in the triterpenoid saponin biosynthesis pathway of *Polygala tenuifolia*. Finally, the function of *CYP716A249* enzyme on oleanolic acid production in *saccharomyces cerevisiae* was confirmed, and the yield of oleanolic acid reached 1.03 mg/L. These results laid a scientific foundation for elucidating the biosynthesis pathway of the saponins in *Polygala tenuifolia* and accelerating the discovery of new drugs related to saponins.

S58-0019

Brain Metabonomics Study of the Anti-depression Effect of Xiaoyaosan on the CUMS-depression Rats by 1H NMR Analysis

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Metabonomics has been successfully used in central nervous system (CNS) disorders in discovering disease mechanisms, demonstrating disease prognosis and therapy efficacy. Often, however, few studies have conducted on brain, the key organ of CNS disorders. In this study, taking Xiaoyaosan (XYS) as the study objectives, we aimed to characterize the diversity and variation of a broad range of metabolites of brains, including both aqueous and lipophilic metabolites, gaining comprehensive insight into the metabolic processes of depression. We established a CUMS-induced depression model. We then extracted both aqueous and lipophilic metabolites of rat brains by a two-phase extraction method, which were subsequently characterized by two differential sequences of 1H nuclear magnetic resonance (NMR), followed by multivariate data analysis. Principal components analysis (PCA) indicated that the metabolic perturbation caused by CUMS was reversed by XYS treatment. In the orthogonal partial least squares-discriminate analysis (OPLS-DA), 6 lipophilic and 9 aqueous metabolites were screened as potential biomarkers. Notably, XYS significantly reversed the abnormality of 5 aqueous and 4 lipophilic metabolites to normal levels, suggesting that XYS synergistically mediated abnormalities of multiple metabolic pathways including D-glutamine and D-glutamate metabolism, alanine, aspartate and glutamate metabolism, taurine and hypotaurine metabolism and arginine and proline metabolism. It is the first report to investigate the anti-depression effect of XYS from the perspective of brain metabolites. In a broad sense, the current study brings novel and valuable insights to evaluate the efficacy of TCM to provide the theoretical basis for further research on the therapeutic mechanism in clinic.

S58-0020

Anti-cancer effects of Avasimibe by inducing cancer cell apoptosis and cell cycle arrest in glioblastoma

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Glioblastoma (GBM), accounting for about 50 % of all glioblastomas, is the most common, malignant, and lethal primary brain tumor in adults. Up to now, there is no effective drug for GBM. Avasimibe, a potent inhibitor of acyl- CoA cholesterol acyltransferase-1 (ACAT-1), was used to removes plaque from blood vessels and treated atherosclerosis. Studies have shown that Avasimibe can be also used for tumor immunotherapy to promote anti-tumor effects by promoting T cell signaling and killing. However, the function and underlying mechanisms of Avasimibe on glioblastomas are still unclear. Here, we found that treatment of Avasimibe in U251 and U87-MG glioblastoma cells inhibited the cell proliferation, decreased the DNA synthesis, and inhibited the colony formation of cells. Avasimibe also increased the apoptotic rate of cells, decreased the mitochondrial membrane potential, inhibited the activity of caspase 3/7, and decreased the protein expression of cleaved-caspase 9, cleaved PARP and Bax in U251 and U87-MG cells. RNA-Sequencing analyses showed that Avasimibe suppressed the expression of CDK2, cyclin E1, CDK4, cyclin D, CDK1, cyclin B1, Aurora A and PLK1 while increased the expression of p53, p21, p27 and GADD45A, which was further validated by Western blot. These results demonstrated that Avasimibe induced mitochondrial dependent apoptosis in glioblastoma cells which was associated with arresting the cell cycle at G0/G1 phase and G2/M phase by regulating the p53/p21 pathway, p53/GADD45A and Aurora A/PLK1 signaling pathways. Taken together, our findings suggest that Avasimibe might be a promising chemotherapy drug in the treatment of GBM.

S58-0021

Research progress of flavonoids against pulmonary hypertension

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Flavonoids, a class of secondary metabolic products widely found in various plants, are the most studied class of polyphenolic compounds and can be divided into flavonols, flavones, flavanones, isoflavones, catechins, anthocyanins, and chalcone according to their chemical structures. Current researches show that flavonoids exert multiple pharmacological activities including anti-oxidation, anti-inflammatory, cardiovascular protection, anti-tumor, anti-viral and anti-allergic effects. Pulmonary hypertension is a chronically severe cardiopulmonary dysfunction with high mortality. Oxidative stress and inflammatory response play key roles in the pathological process of pulmonary hypertension. The currently marketed drugs mainly target the endothelin receptor pathway, the nitric oxide pathway and the cyclooxygenase pathway. But nearly all of them have disadvantages such as single mechanism, serious side effects and high expenses. As a class of natural products extracted from plants, flavonoids can exert therapeutic effects on pulmonary hypertension from multiple aspects with their significant cardiovascular protective effects, and may have the advantage of slight side effects. Based on the researches at home and abroad in recent years, in this review article we review and summarize the so far acquired knowledge of the most important mechanisms of action of flavonoids to provide new candidates and therapeutic strategies for the treatment of pulmonary hypertension.

S58-0022

Metabolic reprogramming in colon cancer reversed by DHTS through regulating PTEN/SIRT3/AKT/HIF1α mediated signal pathway

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Background: Metabolic reprogramming and hypoxia contribute to the resistance of conventional chemotherapeutic drugs in kinds of cancers. In this study, we investigated the effect of dihydrotanshinone I (DHTS) on reversing dysregulated metabolism of glucose and fatty acid in colon cancer and elucidated its mechanism of action. Methods: Cell viability was determined by MTT assay. Oxidative phosphorylation, glycolysis, and mitochondrial fuel oxidation were assessed by Mito stress test, glycolysis stress test, and mito fuel flex test, respectively. Anti-cancer activity of DHTS *in vivo* was evaluated in Colon cancer xenograft. Hexokinase activity and free fatty acid (FFA) content were assessed using respective Commercial kits. Gene expression patterns were determined by performing DNA microarray analysis and real-time PCR. Protein expression was assessed using immunoblotting and immunohistochemistry. Results: DHTS showed potent cytotoxicity against colon cancer cells under hypoxia. DHTS decreased the efficiency of glucose and FA as mitochondrial fuels in HCT116 cells, which efficiently reversed by VO-OHpic trihydrate. DHTS reduced hexokinase activity and free fatty acid (FFA) content in tumor tissue of xenograft model of colon cancer. Gene expression patterns in metabolic pathways were dramatically differential between model and treatment group. Increases in PTEN and a substantial decrease in the expression of SIRT3, HIF1α, p-AKT, HKII, p-MTOR, RHEB, and p-ACC were detected. Conclusions: DHTS reversed metabolic reprogramming in colon cancer through PTEN/SIRT3/AKT/HIF1α-mediated pathway.

S58-0023

Dual -functional peptide with defective interfering genes effectively protects mice against avian and seasonal influenza

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Limited efficacy of current antivirals and antiviral-resistant mutations impairs anti-influenza treatment. Here, we evaluate the in vitro and in vivo antiviral effect of three defective interfering genes (DIG-3) of influenza virus. Viral replication is significantly reduced in cell lines transfected with DIG-3. Mice treated with DIG-3 encoded by jetPEI-vector, as prophylaxis and therapeutics against A(H7N7) virus, respectively, have significantly better survivals (80% and 50%) than control mice (0%). We further develop a dual-functional peptide TAT-P1, which delivers DIG-3 with high efficiency and concomitantly exerts antiviral activity by preventing endosomal acidification. TAT-P1/DIG-3 is more effective than jetPEI/DIG-3 in treating A(H7N7) or A(H1N1)pdm09-infected mice and shows potent prophylactic protection on A(H7N7) or A(H1N1)pdm09-infected mice. The addition of P1 peptide, which prevents endosomal acidification, can enhance the protection of TAT-P1/DIG-3 on A(H1N1) pdm09-infected

mice. Dual-functional TAT-P1 with DIG-3 can effectively protect or treat mice infected by avian and seasonal influenza virus.

S58-0024

The use of a novel ginsenoside derivatives 20(S)-Rh2E2 as effective pharmacological intervention for cancer therapy

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Increased energy metabolism is responsible for supporting the abnormally upregulated proliferation and biosynthesis of cancer cells. The key cellular energy sensor AMPK has been identified as a target for ginseng. Accordingly, ginseng or ginsenosides are potentially valuable to the treatment and/or prevention of cancer via the regulation of energy balance. Ginseng saponin (Rh2) is an active constituent of ginseng which consists of different conformational isomers such as 20(*R*)-Rh2 and 20(*S*)-Rh2. Recent *in vitro* findings suggested that 20(*S*)-Rh2 exhibited profound cytotoxicity not only to cancer cells but also the normal counterpart, which limited the clinical relevance of the compound. In this study, the anti-tumor efficacy of a new chemical derivatives of ginsenoside, 20(*S*)-Rh2E2, was investigated. 20(*S*)-Rh2E2 inhibited tumor growth in a lung xenograft mouse model effectively. Most importantly, animal administrated with 20(*S*)-Rh2E2 up to 320mg/kg/day survive with no significant body weight lost upon 7-day treatment. In addition, we revealed that 20(*S*)-Rh2E2 specifically suppressed cancer cell metabolism *via* the inhibition of metabolic enzymes in mitochondrion and repressed the proliferation of LLC-1 cancer cells by inducing S-phase cell cycle arrest. These findings is consistent to the result obtained from previously reported treatment assay using another similar isomers 20(*R*)-Rh2E2. Our results suggested that 20(*S*)-Rh2E2 could be a new and safe anti-metabolic agent by acting as a tumor metabolic suppressor, which provides a molecular tool for in-depth study of tumor metabolism.

S58-0025

The Discovery of Anilide-containing AMPK Modulators by A Rapid Screening Method Based on Computational Docking and Biological Validation

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Adenosine 5'-monophosphate-activated protein kinase (AMPK) is critical to the maintenance of cellular energy balance and homeostasis. As such, AMPK may serve as pharmacological target for the treatment covered a wide scope of diseases. Thus far, many new AMPK activators have been identified, however, most of them indirectly interact with AMPK, implying the problem of off-target effects. Accordingly, the search for novel modulators directly targeting AMPK with the use effective screening methods is urged. This study reported the successful selection and categorization of a huge amount of compounds from a publicly accessible chemical database, which demonstrated modulatory effect towards AMPK. We discovered a new class of direct AMPK modulator which are compounds containing anilide. The proposed methods combined the use of virtual screening, biological assays, and Lipinski's rule of five assessment, was capable of identifying 13 out of 1,360,000 compounds as AMPK modulators with optimal bioavailability. In addition, the efficacy of these compounds has been analyzed and is found to

be associated with the proton acceptors in their structure, as well as hydrogen bonds with AMPK in the binding site.

S58-0026

Antiplatelet and antithrombotic activities of salvianolic acid A

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Platelets play an important role in pathological thrombosis. Ischemic diseases caused by thrombosis are the main cause of death. Salvianolic acid A is a component of the water-soluble in *Salvia miltiorrhiza*, and the effectives on cardiovascular diseases of salvianolic acid A have been provided. This study was designed to investigate the effects of antiplatelet and antithrombotic of salvianolic acid A. The anti-platelet aggregation effects of salvianolic acid A *in vitro* and *in vivo* were investigated in normal rats. The anti-cerebral ischemia effect of salvianolic acid A was also investigated in rats with thrombotic cerebral ischemia. The results of antiplatelet aggregation *in vitro* showed that salvianolic acid A could inhibit ADP, AA and THR-induced antiplatelet aggregation in a dose-dependent manner, and significantly inhibit THR-induced antiplatelet aggregation with an inhibition rate of 96.83%. The results *in vivo* showed that salvianolic acid A could significantly inhibit ADP, AA and THR-induced antiplatelet aggregation with inhibition rates of 32.70%、54.17% and 8.19%. At the same time, the results of the rat cerebral ischemia model indicated that salvianolic acid A could improve cerebral infarction volume and brain edema, and significantly improve the neurological function. The above results suggest that salvianolic acid A has significantly effect on platelet aggregation and protect against cerebral ischemia induced by thrombus. Therefore, salvianolic acid A has a good application prospect in the prevention and treatment of thrombotic diseases.

S58-0027

Metabolism of aildenafil in vitro in human liver microsomes

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Aildenafil , 1-{[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1Hpyrazolo [4, 3-d] primidin-5-yl) -4- ethoxy phenyl] sulfonyl}-cis-3, 5-dimethylpiperazine, a pyridopyrimidine analogue of sildenafil and vardenafil, is a potent inhibitor of the cyclic guanosine monophosphate (cGMP)-specific phosphodiesterase type V (PDE V). Aildenafil is under development for treatment of erectile dysfunction (ED) and pulmonary arterial hypertension (PAH). The aim of our study was to compare metabolites of aildenafil in vitro in human liver microsomes of male, female and mixed gender. Twenty metabolites have been found in human liver microsomes by UPLC-QTOF-MS spectrometry and identified by UNIFI Scientific Information System. The metabolic pathways of M6 (de-isopropyl amine and hydroxylation), M8 (dehydrogenation and de-methylation), M9 (Dehydrogenation), M14, M15 (hydroxylation and dehydrogenation), M16 (diisopropylamino, hydroxylation and dehydrogenation) were reported for the

first time among the metabolites of the aildenafil. All metabolites were water-soluble molecules without obvious toxic structure. With no differences among metabolites of three group, similar metabolic pathways of aildenafil were observed in the incubations of human liver microsomes from male, female and mixed gender. This suggested that aildenafil had consistent liver metabolic processes in different gender groups. Even though it was used in male in the past, female patients with PAH would not suffer additional side effects due to metabolic processes. In conclusion, these in vitro findings should provide valuable information on possible metabolic behaviors of aildenafil in humans, and further research should be arranged to explore the pharmacokinetics and characteristics of aildenafil in humans.

S58-0028

Pharmacological evaluation of sedative and hypnotic effects of Agarwood in pentobarbital sodium-induced mice

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Insomnia is a mental disorder accompanied with difficulties in falling asleep, and is coupled with daytime consequences such as fatigue and poor attention. It has a close connection with other mental disease, for example, depression and anxiety. Agarwood is a traditional herb which is regarded as tranquilizer for years, whereas underlying pharmacological mechanism is still unclear. Incense of Agarwood is unique for the insomnia treatment. In this study, insomnia mice is induced by intraperitoneal injection of p-chlorophenylalanine (300 mg/kg) for two consecutive days following administration of Agarwood incense (0.3g, 0.5g) for seven days. We found incense of Agarwood has little influence on the locomotor activity of untreated mice. However, it promotes the duration period of sleeping (45mg/kg). The sedative-hypnotic effect is investigated by behavior detection and pentobarbital-induced sleeping assay. Incense of Agarwood exhibits obvious sedative effects by improving individual characteristics and locomotor activity. Also, both nocturnality in night and depression are alleviated by Agarwood. Furthermore, incense of Agarwood shows hypnotic effect by shortening the sleep latency and prolonging the sustained period sleep in mice induced by pentobarbital sodium. These results indicate incense of Agarwood exerts sedative-hypnotic effect through improving the ability of exercise, alleviating depression and promoting sleep of insomnia mice.

S58-0029

The role of pinocembrin in t-PA thrombolysis induced hemorrhagic transformation

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Hemorrhagic transformation (HT) is a frequent complication of ischemic stroke, especially after thrombolytic therapy, and is associated with increased stroke morbidity and mortality. Tissue plasminogen activator (t-PA), as the only FDA-proved drug, is underutilized in ischemic stroke, because of its limited therapeutic window and hemorrhagic complications. Because of the pathomechanism and

therapeutic target are not clear, there are no effective drugs to decrease the incidence of HT. Pinocembrin (PCB) is a natural flavonoid compound and has shown neuroprotective effects in animal ischemic stroke models. In this study, we investigated the role of PCB in t-PA thrombolysis-induced HT in rat thromboembolic stroke model. t-PA was administrated 6 hours after ischemia and PCB (5, 10 and 20 mg/kg) was given 5 mins before t-PA administration. Infarct volume, neurological score and hemoglobin content were evaluated at 24 hours after ischemia. Evans blue leakage was used to detect blood-brain barrier (BBB) permeability. The results showed that treatment with t-PA at 6 hours after ischemia aggravated brain injury and increased the risk of HT. Pretreatment with PCB decreased the infarct volume and brain water content, improve neurological function. In addition, the results of hemoglobin and evans blue leakage showed that PCB could protect the BBB permeability and reduce the occurrence of HT. Among these doses, 10 mg/kg is most effective. In conclusion, these results demonstrate that combination PCB with t-PA protects against cerebral ischemia, reduces the occurrence of HT induced by t-PA thrombolysis. Thus, PCB may be a potential therapeutic drug for t-PA induced HT.

S58-0030

Nrf2 pathway contributed to the anti-inflammatory effect of NC-8, a new compound isolated from Nardostachys chinensis

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Nardostachys chinensis belongs to the Valerianaceae family and is a small, endangered, and perennial herb.NC-8 is a new compound isolated from N. chinensis and the pharmacy activity of NC-8 is unknown. In this study, we found that NC-8 suppressed the inflammatory responses of RAW264.7 cells induced by lipopolysaccharide (LPS) by inhibiting the proteins and mRNA expressions of inducible nitric oxide synthase (iNOS), and reducing the level of NO (Fig.4) but had no effect on cyclooxygenase-2 (COX-2) expression (Fig.1), implying that NC-8 may have a different anti-inflammatory mechanism compared with nonsteroidal anti-inflammatory drugs (NSAID). A mechanistic study indicated that the anti-inflammatory effect was associated with the induction of antioxidant and detoxification enzymes, including heme oxygenase-1 (HO-1) and NAD(P)H dehydrogenases 1(NQO1) at protein and mRNA level.

S59-0001

Outer membrane vesicles as cancer vaccine platform

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Surgery, chemotherapy, radiotherapy, and hormone therapy are common anti-tumor therapeutic approaches. However, the non-specific targeting of cancer cells has made these approaches non-effective and even damaging healthy cells and tissues. For example, adverse effects of chemotherapy can include hair loss, diarrhea, pain, nausea and vomiting, as well as blood related disorders. It is urgent to develop cancer cell targeted drug delivery systems to minimize the dosage utilized for therapy as well as reduce drug leakage to normal non-cancer cells.
Outer membrane vesicles (OMVs) are released by nearly all Gram-negative bacteria and some Gram-positive bacteria. OMVs are nanoscale (~100 nm), non-replicative, highly stable proteoliposomes that contain many of the immunogenic surface-associated components of their parent bacterium. These characteristics make OMVs good candidate for vaccine development. We will modify the parental bacteria, i.e. disrupting the periplasmic crosslinking, to improve the production of OMVs. Furthermore, OMVs will be decorated with cancer antigens to activate the immune system using membrane proteins or lipoproteins as display platform. Also, we will target the OMVs to cancer cells by presenting EGFR ligand on the surface of the OMVs, so that the OMVs will target to cancer cells that overexpress EGFR. Together, our study will develop an OMV-based vaccine platform not only facilitate cancer therapy, but also could be used in infection prevention.

S59-0002

Virotherapy-recruited PMN-MDSC infiltration of mesothelioma blocks antitumor CTL by IL-10-mediated dendritic cell suppression

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Oncolytic virotherapy (OV) represents a promising treatment of solid tumors but its clinical efficacy is often dampened by tumor microenvironment (TME). While a lower frequency of PMN-MDSCs is associated with a higher cytotoxic T lymphocytes (CTLs) response, the underlying mechanism of the blockade of eliciting such antitumor CTLs remains incompletely understood. Here, we show that oncolysis of mesothelioma by modified vaccinia Tiantan (MVTT) induces damage-associated molecular patterns exposure. Although MVTT leads to regression of established mesothelioma dose-dependently, antitumor CTLs are rarely induced. Mechanistically, MVTT virotherapy generates C-X-C chemokines that recruit CXCR2-expressing polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs) into tumor microenvironment, where they suppress dendritic cells (DCs) by producing IL-10 and halt CTL responses. During the virotherapy, however, depletion of PMN-MDSCs but not of monocytic MDSCs results in the induction of potent CTLs, which are essential for immune surveillance and curing of malignant mesothelioma. Our findings suggest that vaccinia virotherapy may combine strategies that prevent the chemotactic recruitment of PMN-MDSCs, block their suppression on DCs or deplete PMN-MDSCs in order to induce potent CTLs for tumor eradication.

S59-0003

Related proteins affecting PD-1/PD-L1 inhibitors efficacy

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Cancer-related diseases has been always the major threat for human health. In recent years, PD-1/PD-L1 therapy has made great progress in dozens of cancers such as melanoma, non-small cell lung cancer and so on. However, only a small proportion of patients responded to monotherapy of anti-PD-1/PD-L1 agents. This review will highlight several proteins related to PD-1/PD-L1 axis, including TIM-3, CMTM6 and PTPN2. Studies have shown that high expression of Tim-3 is associated with T cell depletion and its up-regulation inhibits the continued action of immune checkpoints inhibitors. CMTM6 is a key regulator of PD-L1. It can reduce the levels of PD-L1 ubiquitination and protects PD-L1 from lysosomes degradation. PTPN2(TCPTP) is as tumor suppressor in human bodies, and it can influence efficacy of immunotherapy by interferon-γ signal. These proteins were all closely related to PD-L1 expression and the low efficiency of PD1/PD-L1 axis treatment. Further studies on the regulation of PD-1/PD-L1 axis by these molecules are necessary to find new predictive immune checkpoints, and to provide new strategy for targeting PD-1/PD-L1 therapy. (Acknowledgments: This work was supported by Macao Science and Technology Development Fund Project No: 082/2013/A3, 082/2015/A3, 0003/2018/A1, 130/2017/A3 and 046/2016/A2).

S60-0001

Cocoa butter production using metabolically engineered yeasts

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Cocoa butter (CB) extracted from cocoa beans is the main raw material for chocolate production. However, growing chocolate demands and limited CB production have resulted in a shortage of CB supply. CB is mainly composed of three different kinds of triacylglycerols (TAGs), POP (C16:0-C18:1-C16:0), POS (C16:0-C18:1-C18:0) and SOS (C18:0-C18:1-C18:0). The storage lipids of yeasts, mainly TAGs, also contain relative high-level of C16 and C18 fatty acids and might be used as CB-like lipids (CBLs, mainly POP, POS and SOS) producer, but CBLs are not the major TAG forms in yeast. We selected some cocoa genes or other plant genes potentially responsible for CB biosynthesis from plant genomes and expressed them in *S. cerevisiae*, and the relative CBL contents in three of the yeast strains harboring cocoa genes increased 190%, 230% and 196% over the control strain, respectively. Additionally, one of the three yeast strains had a 2.25-fold increased TAG content and 6.7-fold higher level of CBLs compared with the control strain. However, the highest CBL composition of these yeast strains is less than 5.4%. In order to find other yeast hosts for CBL production, we compared CBL production ability of six different yeasts, including one non-oleaginous yeast strain, *Saccharomyces cerevisiae* CEN.PK113-7D, and five oleaginous yeast strains, *Trichosporon oleaginosus* DSM11815, *Rhodotorula graminis* DSM 27356, *Lipomyces starkeyi* DSM 70296, *Rhodosporidium toruloides* DSM 70398 and *Yarrowia lipolytica* CBS 6124, in nitrogen-limited medium. Under the same growth conditions, we found *T. oleaginosus* can produce more TAGs than the other five yeasts. Besides, *T. oleaginosus* produced 29.8% potential CBLs at levels of 378 mg TAGs/g dry cell weight, hinting that this yeast may have potential as a CBL production host after further metabolic engineering in future.