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BIOCHEMISTRY AND MOLECULAR BIOLOGY

16th INTERNATIONAL CONFERENCE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

SIGNALLING PATHWAYS IN DEVELOPMENT, DISEASE AND AGING

Abstract Book

JULY 17–21, 2016 | VANCOUVER, BC, CANADA

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CONCURRENT SESSIONS

16th INTERNATIONAL CONFERENCE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

SIGNALLING PATHWAYS IN DEVELOPMENT, DISEASE AND AGING

Plenary Sessions

JULY 17-21, 2016 | VANCOUVER, BC, CANADA

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PLENARY SESSION 1 | PL01

PL01.01

PLENARY SESSION 01 - CELL DEATH & AGING
July 17, 2016 17:30 – 19:30

Telomeres, Telomerase and Aging

Peter M. Lansdorp
European Research Institute for The Biology of Ageing, University Medical Center Groningen, Groningen, BC, Netherlands

Abstract: Whereas a single double strand break needs to be recognized and repaired before cells are allowed to divide, all 92 chromosome ends in a human cell need to be "sheltered" from the DNA damage response. How telomeres avoid activation of signaling pathways is hotly debated with a consensus being that at least 13 TTAGGG repeats, and associated "shelterin" proteins are needed for proper telomere "capping". Telomere repeats are added to the ends of chromosomes by the reverse transcriptase telomerase. Telomerase activity is readily detectable in extracts from human hematopoietic cells, but is insufficient to maintain the telomere length in most leukocytes with proliferation *in vitro* and with age *in vivo*. On average leukocyte telomeres shorten at a rate of 30-100 bp per year but the average telomere length at any given age varies over a broad range and is longer in females than in males. Telomere loss may limit the proliferation of stem cells and T lymphocytes and act as a tumor suppressor mechanism in those cells. Strikingly, patients that are haplo-insufficient for telomerase genes invariably show very short telomeres suggesting that telomerase levels regulate the telomere attrition rate. Such patients are at high risk of stem cell failure and, paradoxically, often develop tumors. Telomerase activity is frequently upregulated in tumors by either amplification of the TERT gene or mutations in the TERT promoter. Further studies are needed to develop a better understanding of the role of telomeres and telomerase in normal biology and aging.

PL01.02

PLENARY SESSION 01 - CELL DEATH & AGING
July 17, 2016 17:30 – 19:30

Epigenomic Targets in Cancer and Aging

Karl Riabowol
University of Calgary, Alberta, AB, Canada

Abstract: Epigenomic Targets in Cancer and Aging

PL01.03

PLENARY SESSION 01 - CELL DEATH & AGING
July 17, 2016 17:30 – 19:30

Pores of No Return: How Bcl-2 Family Proteins Regulate Programmed Cell Death.

David Andrews
Biological Sciences, Odette Cancer Program, Sunnybrook Research Institute, Toronto, Canada

Abstract: Direct physical interactions between Bcl-2 family proteins determine cell fate by regulating a form of programmed cell death called apoptosis. Interactions between pro- and anti-apoptotic proteins enable integration of survival and death signals through a funneling down process called mutual sequestration that ultimately regulates permeabilization of the outer mitochondrial membrane by the pro-apoptotic proteins Bax and Bak. Tumor initiation, progression and resistance to chemotherapy rely on cancer cell survival by bypassing apoptosis establishing Bcl-2 family proteins as potential therapeutic targets. The recent FDA approval of the Bcl-2 inhibitor Venetoclax underscores the importance of targeting these proteins. Thus, understanding how the Bcl-2 family regulates cell death provides the rationale for new drug development in cancer. By measuring the interactions between Bcl-2 family proteins in vitro and in live cells we have discovered heretofore unknown binding and activation sites that are therapeutically accessible. I will describe our new model for how Bcl-2 proteins regulate apoptosis as well as current progress in the development and testing of novel methods to guide screening for the next generation of pharmaceuticals that target the Bcl-2 family. The implications of these methods for measuring protein interactions in live cells and for drug discovery in diseases other than cancer will also be discussed.

PLENARY SESSION 2 | PL02

PL02.01

PLENARY SESSION 02 – CANCER CAUSES AND PROGRESSION
July 18, 2016 08:30 – 10:00

Nutritional Risk Factors for Colon and Breast Cancers and for Multiple Sclerosis

Harald Zur Hausen
Deutsches Krebsforschungszentrum, Heidelberg, Germany

Abstract: Red meat consumption has been considered in many prospective epidemiological studies as a major risk for colon cancer. Recently attempts have been published to identify specific infectious factors in red meat contributing to this risk (1-3). The available data point at specific risks after long-time consumption of meat from Eurasian dairy cattle. Breast cancer incidence is high in most countries with high incidence of colon cancer. Yet, in some regions remarkable differences exist: in Japan and Korea breast cancer occurs less frequently than colon cancer, in India breast

cancer exceeds the rate of colon cancer. In additional countries (China and Bolivia) breast cancer incidence is low, corresponding to low consumption of cow milk. A correlation seems to exist between dairy cattle milk consumption and breast cancer incidence (2). We isolated 22 small novel single-stranded circular DNA molecules from cattle sera and dairy products, belonging into three different groups. Human sera were negative for these agents. Autopsy material, however, from two patients with multiple sclerosis contained agents related to milk isolates. All isolates tested in human cells are genetically active. Although suggestive, the available data do not provide definite evidence for a role of their in human diseases.

- zur Hausen H. Int J Cancer. 2012; 130: 2475-83.
- zur Hausen, H., de Villiers, E. M. Int J Cancer. 2015; 137: 959-967.
- zur Hausen, H. Nature Rev. Clinical Oncology, 2015; 12: 569-70.

PL02.02

PLENARY SESSION 02 – CANCER CAUSES AND PROGRESSION
July 18, 2016 08:30 – 10:00

Genomic Analysis for Personalized Medicine

Steven Jones
Genome Sciences Centre, BC Cancer Agency, Vancouver, BC, Canada

Abstract: Presented of on behalf of the BC Cancer Agency Personalized Oncogenomics project. Genomic analysis is being widely investigated to support cancer treatment decision-making. The Personalized Oncogenomics (POG) project enrolls patients with metastatic cancers for which standard chemotherapy regimens fail or do not exist. We are performing whole genome and transcriptome sequencing of a fresh tumour biopsy sample and whole genome sequencing of peripheral blood using the Illumina DNA sequencing platform. Bioinformatics approaches are used to identify genes with somatic alterations, copy number variants, regions of loss of heterozygosity, structural variants, and expression changes to build an individual somatic molecular profile. A de-novo assembly of the tumour genome and transcriptome is also conducted to detect complex structural re-arrangements and gene fusions. This is followed by intensive pathway analysis and literature searches to identify potential therapeutic options. POG has consented over 500 patients and sequenced and analyzed samples from over 400 patients representing more than 30 different tumour types. The project represents a computational challenge in terms of the large amount of data that needs to be processed rapidly and within a clinically relevant timeframe. We also require a computational framework to correlate observed genetic lesions with known cancer driving events and drug sensitivities. Ultimately, many inferences will be made through comparison to previously analyzed cancer genomes and their associated clinical responses.

PL02.03

PLENARY SESSION 02 – CANCER CAUSES AND PROGRESSION
July 18, 2016 08:30 – 10:00

Can Tumor Growth Be Controlled by Controlling Lipid Synthesis?

Beatriz L. Caputto
Departamento De Quimica Biologica, Facultad De Ciencias Quimicas, CIQUIBIC (CONICET) - Universidad Nacional de Cordoba, Cordoba, Argentina

Abstract: Lipids are the quantitatively most important molecular species of every cell membrane. Cells actively involved in proliferation demand massive membrane biogenesis and hence, lipid biosynthesis rates must be higher than in cells that are neither dividing nor actively growing. However, the nature of the regulatory events underlying such processes is poorly understood. We have shown that the protein c-Fos is actively involved in these regulatory events. c-Fos, a member of the AP-1 family of inducible transcription factors, has been hypothesized to participate in promoting growth by regulating the expression of cell-growth-related genes. We established that c-Fos is also capable of regulating growth by a non-genomic activity: it activates the biosynthesis of lipids at the endoplasmic reticulum. Lipid-synthesis activation by c-Fos is accomplished through a physical association between its N-terminal domain and the enzymes it modulates whereas rate activation of reactions is promoted through its basic domain (amino acids 139-159). In human brain biopsies, c-Fos expression is at the limit of detection in non-pathological specimens, but is abundantly expressed in all of the >150 tumors examined. In mice, blocking c-Fos expression, or in c-Fos -/- mice, tumor cell proliferation is slowed/halted without substantial changes in the AP-1 content. Only one other protein, Fra-1, was found that contains a c-Fos-homologous basic domain, and interestingly, it also activates lipid synthesis and sustains tumor growth. We are currently examining the capacity of c-Fos and/or Fra-1 fragments to act as negative dominants to control brain and breast tumor cell growth.

PLENARY SESSION 3 | PL03

PL03.01

PLENARY SESSION 03 – SIGNALING IN CELL BIOLOGY & DEVELOPMENT
July 18, 2016 15:45 – 17:15

Molecular Mechanism of Cytokinesis

Thomas D. Pollard
Mcdb, Yale University, New Haven, CT, United States of America

Abstract: We use three complementary approaches to study the molecular basis of cytokinesis. We characterize the structures and biophysical properties the participating proteins to generate hypotheses about the biochemical reactions. Quantitative fluorescence microscopy of live cells expressing fluorescent fusion proteins gives the numbers of each type of protein in whole yeast

cells with one second temporal resolution. High-speed super resolution localization microscopy (FPALM) allows us to image structures at 35 nm resolution in live cells on a time scale of a few seconds. Comparisons of these quantitative measurements in cells with simulations of molecularly explicit mathematical models of the reactions in our biochemical hypothesis usually suggest ways to improve our hypotheses. Our simulations now reproduce the time course of events during the assembly and constriction of the cytokinetic contractile ring as observed in live cells and predict the outcomes of experimental manipulations.

PL03.02

PLENARY SESSION 03 – SIGNALING IN CELL BIOLOGY & DEVELOPMENT
July 18, 2016 15:45 – 17:15

Investigating Signal Transduction in Drosophila: A Powerful Model for Disease Mechanisms

Esther M. Verheyen[1], Jessica Blaquiere[2], Nathan Wray[2]
[1]Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, BC, Canada; [2]Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, Canada

Abstract: Drosophila have been used for decades to study developmental signaling pathways and have been key in revealing molecular functions of human disease and cancer-related genes. For many oncogenic pathways much of the molecular circuitry was elucidated in flies. Drosophila has also emerged as an excellent model for hematopoietic study, within the context of fly's simplified cell lineage. The evolutionarily conserved Homeodomain-Interacting-Protein-Kinase (Hipk) is a potent regulator of proliferation and signal transduction. Elevated levels of Hipk in Drosophila lead to tumour-like masses resembling those found with activated JAK/STAT signaling. A point mutation like those seen in human blood cancers in the Drosophila JAK causes constitutive activation of the pathway and results in blood cell tumours in larvae and adult. We found that Hipk causes tumours through JAK/STAT based on a number of observations. Elevated Hipk in blood cells phenocopies effects seen with hyperactive form of JAK. Furthermore, Hipk induces enhanced proliferation of hemocytes. Reduction of Hipk can suppress the tumorigenic effects of activated JAK. RNAi against Hipk in hemocytes can suppress effects of activated JAK. Lastly, we find that Hipk is required for endogenous JAK/STAT pathway activity, since Hipk is required for expression of a STAT reporter. Thus we provide robust genetic evidence that Hipk is a novel pathway regulator that can induce fly blood tumors. A proximity ligation assay that showed an interaction between Hipk and STAT92E, the Drosophila STAT. Our work shows that Hipk is required for JAK/STAT signaling during normal development and in fly blood cancer.

PL03.03

PLENARY SESSION 03 – SIGNALING IN CELL BIOLOGY & DEVELOPMENT
July 18, 2016 15:45 – 17:15

Charting the Human Centrosome-Cilium Landscape

Laurence Pelletier
Lunenfeld Tanenbaum Research Institute, Toronto, Canada

Abstract: The centrosome is the major microtubule-organizing center (MTOC) in animal cells. Centrosomes control a plethora of cellular processes including the organization of the interphase microtubule network, the assembly of the mitotic spindle and the formation of cilia/flagella. Mitotic cell division relies on the formation of a robust bipolar spindle, which mediates the accurate segregation of genetic material to daughter cells. In mitosis, two centrosomes need to be present, each organizing one of the two spindle poles. Numerical and structural centrosome aberrations can give rise to the formation of abnormal mitotic spindle and genome instability. In vertebrates, cilia fulfill diverse functions critical for embryonic development and the homeostasis of adult tissues. Therefore, defects in cilia biogenesis and function can lead to the onset disease, commonly referred to as ciliopathies including conditions such as blindness, infertility, obesity, mental retardation, situs inversus and polycystic kidney disease. In my presentation I will discuss our work using sub-diffraction imaging coupled with functional proteomics to generate a nanometer functional atlas of the human centrosome-cilium interface and discuss recent progress on our understanding of the molecular mechanism underpinning the assembly and function of these fascinating organelles.

PLENARY SESSION 4 | PL04

PL04.01

PLENARY SESSION 04 – CIRCADIAN RHYTHMS
July 19, 2016 08:30 – 10:00

Molecular Regulation of Sleep and Circadian Rhythms

Michael W. Young
Genetics, Rockefeller University, new york, United States of America

Abstract: We have identified genes that control the circadian rhythms of Drosophila. Interactions among these genes and their proteins set up a network of oscillations within single cells. These oscillations are autonomously generated, are found in most tissues, and establish rhythms in physiology and behavior. This mechanism is conserved within the animal kingdom: similar "clock" genes regulate patterns of sleep and other rhythms in humans. A common form of human insomnia called Delayed Sleep Phase Disorder (DSPD) is characterized by a persistent and intractable delay in the timing of the major sleep episode. A study of several DSPD subjects allowed us to recognize a specific clock gene variant that affects behavioral, physiological and molecular circadian rhythms of carriers under controlled laboratory conditions. Our results

are consistent with the candidate allele encoding a dominant, hyperactive transcription factor that alters sleep and circadian rhythms by lengthening the period of the circadian clock.

PL04.02

PLENARY SESSION 04 – CIRCADIAN RHYTHMS
July 19, 2016 08:30 – 10:00

Synthetic Biology: From a Hybrid Circadian Oscillator to the Generation of Live Images and Clock-based Eidetic Memory

Luis F. Larrondo
Genética Molecular Y Microbiología, Millennium Nucleus for Fungal Integrative and Synthetic Biology, Pontificia Universidad Católica de Chile, Santiago, Chile

Abstract: In the fungus Neurospora crassa, as in other model organisms, synthetic biology based-strategies have been seldom adopted for the study of circadian oscillators. Our current efforts on this matter have focused on examining the plasticity of the Neurospora circadian clock through transcriptional rewiring. This design implies the addition of new positive elements (transcription factors) that are now integral part of a hybrid oscillator (HO) that mixes canonical and new components. Remarkably, this HO free-runs, has a period close to 24 h, is temperature compensated and it is entrainable by external cues. Such an approach is already revealing important insights regarding time-delay mechanisms and alternative design principles compatible with clock function. On the other hand, we have adopted optogenetic approaches to further delve into Neurospora's circadian and light-responses. In doing so, we had the ability to turn this fungus into a "live canvas" on top of which images can be projected causing a bioluminescent biological response that recreates the original image with great precision. Remarkably, since this optogenetic circuit is integrated in the Neurospora circadian regulatory network, the fungus reproduces on subsequent days -in a circadian manner- the image that it had originally "seen", creating an eidetic (photographic) memory effect. Such phenomenon, based on local discrete phase changes, not only will provide new insights on phase responses, but it also allows for the opportunity to ponder on concepts such as vision and memory.
Funding: FONDECYT 1131030 MN-FISB NC120043.

PL04.03

PLENARY SESSION 04 – CIRCADIAN RHYTHMS
July 19, 2016 08:30 – 10:00

Circadian Rhythms in Mammals: Entrainment by Meal Timing

Ralph Mistlberger
Psychology, Simon Fraser University, Burnaby, Canada

Abstract: Daily rhythms in mammals are regulated by a distributed system of circadian pacemakers and oscillators in the brain and throughout the body. A master pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) entrains to the solar day by retinal input, and confers internal temporal order via direct and indirect output pathways to local oscillators driving tissue specific rhythms. SCN control of the daily feeding rhythm appears to play a prominent, if not dominant role as a synchronizing factor for much of the circadian system. Local oscillators in most organs and many brain regions synchronize to daily feeding rhythms, and are reset by shifts of mealtime. Some of these oscillators regulate foraging behavior, ensuring that daily rhythms of behavior and physiology are appropriately synchronized and resonant with daily feeding opportunities. I will provide a current account of what we know, and what we need to learn, about the neural, endocrine and metabolic signaling pathways by which meal timing regulates circadian timing in health and disease.

PLENARY SESSION 5 | PL05

PL05.01

PLENARY SESSION 05 – SIGNALING AND IMMUNE FUNCTION
July 19, 2016 15:45 – 17:15

Exploiting Oxidative Stress in Cancer

Tak Mak
University of Toronto, Toronto, ON, Canada

Abstract: The regulation of oxidative stress is an important factor in both tumor development and responses to anti-cancer therapies. Many metabolic signaling pathways that are linked to tumorigenesis can also regulate the metabolism of reactive oxygen species (ROS) through direct or indirect mechanisms. For example, isocitrate dehydrogenases 1 and 2 (IDH1/2), carnitine palmitoytransferase 1c (CPT1c), PARK-7, estrogen, UDPase, HMGB1, AhR, GCLC(M) etc. High ROS levels are generally detrimental to a cell and trigger its death. As a consequence, the redox status of cancer cells differs from normal cells and cancer cells often exhibit an elevation of ROS. These observations suggest that ROS may constitute a barrier to tumorigenesis. However, ROS can also promote tumor formation by inducing mutations. These contradictory effects have important therapeutic implications for the modulation of ROS as an antitumour strategy. In this presentation, we address the controversial role of ROS in tumor development and in responses to anti-cancer therapies, and elaborate on the idea that targeting the antioxidant capacity of tumour cells has a positive therapeutic impact. The oxidative stress levels are also related to the extent of the DNA damage of a tumor cell. In this context, we have developed two inhibitors that affect cell cycle and apoptosis. We are targeting two mitotic enzymes PLK4 and TTK. PLK4 is known to be involved in centriole duplication and genomic instability whereas TTK is linked to spindle assembly checkpoints. Progress of the clinical trials of these compounds will be also be discussed.

PL05.02

PLENARY SESSION 05 – SIGNALING AND IMMUNE FUNCTION
July 19, 2016 15:45 – 17:15

Integrins Facilitate Fc Receptor Ligation and Src-Family Kinase Activation during Phagocytosis

Spencer Freeman[1], Sergio Grinstein[2]
[1]The Hospital for Sick Children, Toronto, AB, Canada; [2]Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada

Abstract: Integrins facilitate Fc receptor ligation and Src-family kinase activation during phagocytosis.
Spencer A. Freeman and Sergio Grinstein. Cell Biology Program, The Hospital for Sick Children, Toronto, Canada, M5G 1X8.

Phagocytosis is initiated by lateral clustering of receptors, which in turn activates Src-family kinases (SFKs). Activation of SFKs requires exclusion of tyrosine phosphatases from the phagocytic cup. We used single-molecule tracking to investigate how the major phosphatase, CD45, is excluded from sites of contact with the phagocytic target. A 2-dimensional frustrated phagocytosis model was implemented to stabilize the focal plane. The mobility of CD45 increased markedly upon engagement of phagocytic (Fcγ) receptors. While individual CD45 molecules moved randomly in the plane of the membrane, they were displaced from the advancing phagocytic cup by an expanding diffusional barrier. Micropatterning of IgG, the ligand of Fcγ receptors, was used to define the relationship between engaged receptors and the diffusional barrier. Remarkably, the barrier extended well beyond the perimeter of the receptor-ligand interaction zone. Second messengers generated by Fcγ receptor activation were found to activate integrins, which were shown to form the diffusion barrier that excluded CD45. The expanding integrin wave facilitates the "zippering" of Fcγ receptors onto the target and integrates the information from sparse receptor-ligand complexes, coordinating the progression and ultimate closure of the phagosome.

Supported by grant FDN143202 from the Canadian Institutes of Health Research.

PL05.03

PLENARY SESSION 05 – SIGNALING AND IMMUNE FUNCTION
July 19, 2016 15:45 – 17:15

SLAM Family Receptors and Immune Responses

André Veillette, Ning Wu, Huaijian Guo
Molecular Onlcology Laboratory, Institut de recherches cliniques de Montreal (IRCM), Montreal, QC, Canada

Abstract: Natural killer (NK) cells play a key role in anti-tumor and anti-viral immunity, as well as normal immune regulation. Their activation leads to induction of cytotoxicity and production of cytokines such as interferon (IFN)-g, which promote elimination of abnormal or unwanted cells. NK cells are particularly efficient at eliminating hematopoietic cells, in contrast to non-hematopoietic cells. The balance between stimulation of various activating and inhibitory receptors, by ligands that may or may not be present on potential target cells, largely determines whether NK cells are activated and eliminate targets. However, NK cell responsiveness is also modified by prior cues received from surrounding cells, in particular from normal hematopoietic cells. This modulatory influence has been named "education". Recent studies from our laboratory have elucidated the roles and mechanisms of action of various receptors implicated in NK cell activation and education. These include SLAM family receptors, which interact with SAP family adaptors and play a key role in the ability of NK cells to discriminate between hematopoietic and non-hematopoietic target cells. We recently found that one of the SLAM family receptors, SLAMF6, plays a key role in education of NK cells towards non-hematopoietic cells. We also determined the global role of the SLAM family in NK cells by creating a mouse lacking all 6 SLAM family receptors. These data will be presented.

PLENARY SESSION 6 | PL06

PL06.01

PLENARY SESSION 06 – REGULATION OF RNA & PROTEINS
July 20, 2016 08:30 – 10:00

Regulatory RNA

Andrew Fire
Stanford University, Stanford, CA, United States of America

Abstract: Regulatory RNA

PL06.02

PLENARY SESSION 06 – REGULATION OF RNA & PROTEINS
July 20, 2016 08:30 – 10:00

Molecular Chaperones in Protein Folding and Proteostasis Maintenance

F. Ulrich Hartl
Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany

Abstract: The past two decades have witnessed a paradigm shift in our understanding of cellular protein folding. While the three-dimensional structures of functional proteins are determined by their amino acid sequences, it is now firmly established that in the cell many proteins depend on molecular chaperones to reach their folded states efficiently and on a biologically relevant time scale. Assistance of protein folding is provided by different types of chaperone which act to prevent misfolding and aggregation, often in an ATP-dependent mechanism. Molecular chaperones also cooperate with the degradation machinery (ubiquitin-proteasome system and autophagy) in the removal of terminally misfolded proteins. Once folded, many proteins continue to

require chaperones to retain their functional states, especially under conditions of cell stress. Failure of the chaperone network to maintain proteostasis, i.e. the conformational integrity of the cellular proteome, facilitates the manifestation of diseases in which proteins misfold and are deposited as aggregates, such as Parkinson's and Huntington's disease. Proteostasis undergoes a decline during aging, presumably explaining why age is a major risk factor of neurodegenerative pathologies. I will briefly summarize the molecular chaperone concept and then discuss the role of the chaperone network under normal conditions and in models of aggregate deposition disease.

PL06.03

PLENARY SESSION 06 – REGULATION OF RNA & PROTEINS
July 20, 2016 08:30 – 10:00

Dysregulation of mRNA Translation by Signalling Pathways in Cancer and Neurodevelopmental Diseases

Nahum Sonenberg
Biochemistry, McGill University, Montreal, Canada

Abstract: Dysregulation of mRNA Translation by Signalling Pathways in Cancer and Neurodevelopmental Diseases

PLENARY SESSION 7 | PL07

PL07.01

PLENARY SESSION 07 – CANCER SIGNALING PATHWAYS
July 21, 2016 08:30 – 10:00

Reversing the Paradigm: Protein Kinase C as a Tumor Suppressor

Alexandra Newton
University of California, San Diego, La Jolla, AL, United States of America

Abstract: Protein kinase C (PKC) isozymes transduce the myriad of signals resulting from receptor-mediated hydrolysis of phospholipids, playing critical roles in diverse cellular functions. Its activity must be precisely tuned for cellular homeostasis and too much, or not enough, activity results in pathophysiologies. The discovery in the 1980s that PKCs are receptors for the potent tumor promoting phorbol esters led to the dogma that activation of PKC by phorbol esters promotes tumorigenesis. However, clinical trials with PKC inhibitors have not have not only failed but, in some cases, resulted in worsened outcome. Analysis of approximately 50 mutations identified in human cancers reveals that most mutations are loss-of-function and none are activating. Loss-of-function mutations occur in all PKC subgroups and occur by disrupting diverse mechanisms: they impede second-messenger binding, prevent phosphorylation, or inhibit catalysis. Thus, PKC functions as a tumor suppressor and its loss of confers a survival advantage. In striking contrast, enhanced activity of PKC is associated with degenerative diseases, with gain-of-function variants in one isozyme of PKC, PKCalpha, identified in Alzheimer's disease. Our results reveal that therapeutic strategies for cancer should focus on restoring, rather than inhibiting, PKC activity in cancer. and suggest that inhibitors for PKC could be repurposed for Alzheimer's Disease.

PL07.02

PLENARY SESSION 07 – CANCER SIGNALING PATHWAYS
July 21, 2016 08:30 – 10:00

Met Receptor Tyrosine Kinase; Feedback Inhibition, Rewiring and Mechanisms of Resistance

Morag Park, Paula Pinto Coehlo, Jennifer Knight, Tunde Golenar, Colin Radcliffe, Charles Rajadurai, Veena Sangwan, Yaakov Stern, Crista Thompson, Dongmei Zuo
Departments of Biochemistry and Oncology, Rosalind and Morris Goodman Cancer Research Centre, McGill University, Montréal, Canada

Abstract: The Met receptor tyrosine kinase (RTK) and its ligand, hepatocyte growth factor (HGF), are potent mediators of epithelial-mesenchymal transition and invasive growth. It is now evident that Met plays a prominent role in many solid cancers by apparently distinct mechanisms. Ligand-independent Met activation, through Met amplification, is a driver for a subset of prevalent and hard to treat cancers, (gastric, non-small cell lung) and represents a mechanism of resistance to targeted therapies against other RTKs such as EGFR family members. In other cancers, MET promotes cell adaptation to an adverse microenvironment, and HGF is a prominent microenvironmental factor that confers resistance to targeting agents. HGF-dependent activation of Met plays a prominent role in cancer associated EMT, enhanced stemness and cell survival. In this context, Met does not act as an oncogenic driver but is associated with resistance to standard therapies and poor outcome in multiple cancers. HGF activation of Met is tightly regulated involving subsequent degradation of Met as a predominant mechanism for signal termination yet amplified and mutant Met RTKs escape degradation through multiple mechanisms. Met small molecule tyrosine kinase inhibitors (TKIs) are currently in clinical trials and resistance to Met TKIs is considered a challenge to targeted therapies. We have addressed mechanisms of resistance in gastric cancer carrying an amplification of the *MET* gene that are dependent on Met for proliferation and are sensistive to Met inhibition.

PL07.03

PLENARY SESSION 07 – CANCER SIGNALING PATHWAYS
July 21, 2016 08:30 – 10:00

Destabilization of Ras via Targeting the Wnt/Beta-Catenin Pathway Is a Potential Therapy for Colorectal Cancer

Kang-Yell Choi
Biotechnology/ Translational Research Center For Protein Function Control, Yonsei University, Seoul, Korea, Republic of

Abstract: The Wnt/β-catenin and Ras/extra cellular signal-regulated kinase (ERK) pathways synergistically interact in the tumorigenesis of colorectal cancer (CRC), and mutational activation of each of these pathways, such as by adenomatos polyposis coli (APC) or K-Ras mutation, weakly promoted tumorigenesis. However, our understanding for the interaction of the two pathways in the tumorigenesis is poor.
Activation of the ERK pathway within 5 minutes of Wnt ligand stimulation, indicated direct interaction of the two pathways. We found that Ras as well as beta-catenin were subjected to proteasomal degradation via inhibition of the Wnt/beta-catenin pathway. Ras degradation was achieved/occurred via GSK3beta-mediated phosphorylations at Thr-144 and Thr-144 and subsequent recruitment of beta-TrCP E3-linker to the phosphorylation sites. Accordingly, loss of APC which resulted in increments of both beta-catenin and Ras. The initiation and progression of CRC were critically increased by mutations of both APC and K-Ras which also frequently occur as high as 90% and 40-50% of CRC, respectively. K-Ras mutation alone did not significantly induce tumorigenesis, but additional APC mutation which stabilizes mutant K-Ras resulted in liver metastasis involving activation of cancer stem cells. A pathological significance of the stabilizations of β-catenin and Ras as well as their mutations in tumorigenesis was also supported by high levels of both β-catenin and Ras proteins in the majority of tumor tissues of CRC patients. In this presentation, I will also describe our small molecular approach to control CRC and other cancers with activated Wnt/beta-catenin and Ras pathways via targeting the Wnt/beta-catenin pathway.

PLENARY SESSION 8 | PL08

PL08.01

PLENARY SESSION 08 – MEMBRANE PROTEINS & CHANNELS
July 21, 2016 15:30 – 17:00

SLC4A11- A Membrane Transport Protein Causing Corneal Blindness

Joseph R. Casey
Biochemistry, University of Alberta, Edmonton, AB, Canada

Abstract: SLC4A11 is an integral membrane protein of the basolateral membrane of the corneal endothelium. Mutations of SLC4A11 give rise to corneal endothelial dystrophies (Fuchs endothelial corneal dystrophy, congenital hereditary endothelial dystrophy and Harboyan syndrome), marked by fluid accumulation in the corneal stroma, which profoundly affect vision quality. SLC4A11 normally moves water and ammonia from endothelial cells. This talk will provide evidence for SLC4A11 function. To date about 60 point mutants of SLC4a11 have been identified. Many of these cause the protein to mis-fold, leading to retention of the protein in the endoplasmic reticulum, the site of membrane protein biosynthesis. We have characterized the phenotype of all identified mutants, to identify those retained in the ER. These ER-retained mutants are targets for therapeutics aimed at folding correction to rescue the protein to the plasma membrane. We screened drugs and identified glafenine as a corrector of SLC4A11 folding defects. Thus, corneal dystrophy patients with ER-retained SLC4A11 are candidates for folding correction therapy. Finally, SLC4A11 is a member of the SLC4 family of transport proteins. We made a homology model for SLC4A11 on the basis of a recently-reported crystal structure (3.5 Å resolution) for Band 3 (SLC4A1). The homology model provides a rationale for the observed phenotypes of SLC4A11 mutations.

PL08.02

PLENARY SESSION 08 – MEMBRANE PROTEINS & CHANNELS
July 21, 2016 15:30 – 17:00

Mechanisms and Pathologies of Protein Transport into the Endoplasmic Reticulum

Richard Zimmermann
Medical Biochemistry & Molecular Biology, Saarland University, Homburg, Germany

Abstract: The endoplasmic reticulum (ER) of mammalian cells forms a vast membrane network and plays central roles in protein biogenesis and calcium signaling. The protein biogenesis function involves different receptor systems for targeting of nascent or newly-syntheszied precursor polypeptides to the ER and an aqueous polypeptide-conducting channel in the ER membrane, which facilitates membrane insertion and translocation of precursor polypeptides. The channel is formed by the heterotrimeric Sec61 complex. Sine the store- and receptor-controlled calcium release function of the ER requires a steep ER to cytosol calcium gradient, gating of the calcium-permeable Sec61 channel is tightly controlled by various allosteric effectors. The ER proteins Sec62, Sec63, and BiP facilitate Sec61 channel gating from the closed to the open state for protein transport in a precursor specific manner. BiP with its co-chaperones ERj3 and ERj6, Ca^{2+}-Sec62, and Ca^{2+}-Calmodulin contribute to Sec61 gating from the open to the closed state. Our recent work identified polycystic liver disease (PLD) as a protein transport disease and suggests that the patho-physiological effects of *SEC61A1* missense mutations in diabetes or common varaiable immune deficiency (CVID), loss of ERj6 function mutations in diabetes, and *SEC62* over-expression in tumors of the lung and prostate are linked to the role of these proteins in cellular calcium homeostasis.

PL08.03

PLENARY SESSION 08 – MEMBRANE PROTEINS & CHANNELS
July 21, 2016 15:30 – 17:00

Regulation of Voltage-Gated Sodium Channels by Calcium Ions and Auxiliary Subunits

Filip Van Petegem
Biochemistry and Molecular Biology, University of British Columbia, Vancouver, Canada

Abstract: Voltage-gated sodium channels are integral membrane proteins that can rapidly depolarize the plasma membrane. They are responsible for the action potential in many excitable cells, including neurons and cardiac myocytes. The cardiac sodium channels are targets for a multitude of mutations that cause Long-QT and Brugada syndromes, two types of inhereted arrhythmias. They consists of two subunits: a pore-forming alpha-subunit, which forms the ion conduction pathway, and an auxiliary beta-subunit, which can have profound effects on protein expression, pharmacology, and electrophysiological properties. Sodium channels undergo very complex regulatory events, and have the property to inactivate, a process whereby channel activity is shut down. The rapid inactivation is mediated by distinct cytosolic components that form hot spots for disease-causing mutations. Here we describe high-resolution crystal structures of the auxiliary subunits, and of the inactivation machinery. Coupled with electrophysiological assays, we describe mechanisms that can regulate channel inactivation, and the mechanisms through which disease-associated mutations can affect channel function.

16th INTERNATIONAL CONFERENCE OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

SIGNALLING PATHWAYS IN DEVELOPMENT, DISEASE AND AGING

Concurrent Sessions

JULY 17–21, 2016 | VANCOUVER, BC, CANADA

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CONCURRENT SESSION 01 | CS01
METABOLIC SIGNALING AND CHANNELS

CS01.01

CONCURRENT SESSION 01: METABOLIC SIGNALING AND CHANNELS
July 18, 2016 10:45 – 12:15

Insulin Signals Leading to GLUT4 Translocation in Muscle Cells

Amira Klip
Cell Biology Program, The Hospital for Sick Children, Toronto, ON, Canada

Abstract: Insulin stimulates glucose entry into skeletal muscle, primarily through the rapid mobilization of vesicles containing GLUT4 glucose transporters, from an intracellular endosomal pool to the plasma membrane. This endosomal pool is specific of skeletal muscle, heart and adipose cells, and is distinct from but in dynamic exchange with recycling endosomes. Insulin promotes the rate of externalization of GLUT4 vesicles from this 'specialized pool', through a signalling cascade downstream of PI3-kinase that bifurcates to activate Akt2 and Rac1. Both signalling arms are required for full mobilization of GLUT4 vesicles. We will describe the signals elicited by Akt2 and how they intersect downstream with the Rac1-instructed reorganization of cortical actin filaments. Specifically, Akt2 leads to phosphorylation of the Rab-GAP AS160/TBC1D4, thereby inactivating its GAP activity and enabling activation of its Rab targets to prevail. In skeletal muscle cells, these GTPases are Rab8A and Rab13. We will show how GTP-loaded Rab8A engages the processive myosin MyoVa to mobilize GLUT4 out of the perinuclear compartment. Once in the peripheral region of the cell, GLUT4 itself binds to the actin filament-binding protein alpha-actinin4 (ACTN4). This protein in turn binds to the linker protein MICAL-L2 in response to unfolding of the latter by GTP-loaded Rab13. In this way, we envisage GLUT4 vesicles to latch on to the cortical actin network and be positioned for immediate fusion with the plasma membrane. This latter step is mediated by formation of a SNARE complex between VAMP2 on the GLUT4 vesicle and the plasma membrane t-SNAREs syntaxin4 and SNAP23.

CS01.02

CONCURRENT SESSION 01: METABOLIC SIGNALING AND CHANNELS
July 18, 2016 10:45 – 12:15

Cell Signaling Pathways in Cardiac Autophagy

Sergio Lavandero
Universidad de Chile, Santiago, Chile

Abstract: Cell Signaling Pathways in Cardiac Autophagy

CS01.03

CONCURRENT SESSION 01: METABOLIC SIGNALING AND CHANNELS
July 18, 2016 10:45 – 12:15

Physiological Implications of Anoctamin 1, a Calcium-Activated Chloride Channel

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Abstract: Anoctamin 1 (ANO1 or TMEM16A) was cloned to be a candidate for CaCC. ANO1 is activated by intracellular Ca2+, which is also voltage dependent. ANO1 is expressed in epithelia of salivary glands, pancreas, kidney, pulmonary airways, the retina, and sensory neurons where endogenous CaCC currents were found. ANO1 is highly expressed in dorsal-root ganglion (DRG) neurons, suggesting a role in nociception. ANO1 is activated by heat over 44oC. ANO1 is highly co-expressed with TRPV1, a marker for nociceptors, suggesting the involvement in nociception. Ano1-deficient mice specifically in DRG neurons were generated. Ano1-deficient mice showed reduced responses to painful heat. Thus, Ano1 plays an important role in mediating nociception in sensory neurons. Cl- secretion is important for protection of intestinal epithelia. Whether CaCC plays a role for the Cl- secretion in GI tracts is not known. Two different mutant mice that lack Ano1 in small intestine and large intestines were generated. When Ano1 is abolished in small and large intestines, carbachol-induced Cl- conductance was significantly reduced in duodenum, jejunum and proximal colon. In addition, the colon of Ano1 deficient mice was edematous. Furthermore, when colitis was induced by dextran sodium sulfate (DSS), Ano1-deficient mice developed severe colitis in colon. These results clearly suggest that ANO1 plays an active role in secreting Cl- in intestines. In addition, ANO1 plays a critical role in testosterone-induced benign prostate hyperplasia. Thus, in this talk, the recent data revealing the role of ANO1 in benign prostate hyperalgesia will be presented. Supported by NRF (No. 20110018358) and BK21+ program

CS01.04

CONCURRENT SESSION 01: METABOLIC SIGNALING AND CHANNELS
July 18, 2016 10:45 – 12:15

Influence of Parkia Biglobosa Protein Isolate on Biomarkers of Oxidative Stress in Brain and Testes of STZ-Diabetic Rats

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Abstract: *Parkia biglobosa* is believed to possess antioxidant activity that may exert modulatory effects on diabetes and diabetic complications. This study investigated the modulatory potential of *Parkia biglobosa* protein isolate (PBPi) on serum testosterone

(sTT) levels, as well as its influence on biomarkers of oxidative stress in the brain and testes of streptozotocin-induced diabetic rats. Animals were made diabetic with a single intraperitoneal administration of streptozotocin (STZ; 60 mg/kg body weight). PBPi (200 or 400 mg/kg body weight) was given orally by gavage or insulin (5 U/kg, i.p.) was administered daily to STZ-induced diabetic rats for 28 days. The results revealed a significant elevation in thiobarbituric acid reactive substance (TBARS) levels in the brain and testes of diabetic rats. This was closely associated with the concomitant reduction in levels of sTT and reduced weight in the testes. It was also closely associated a noticeable decline in the glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT), as well as total glutathione (total GSH) levels in the brain and testes of diabetic rats. Interestingly, treatment with PBPi efficiently prevented the alterations witnessed in the serum sTT and also ameliorated various alterations in the biomarkers of oxidative stress (TBARS, total GSH, GST, SOD and CAT) in the brain and testes of diabetic rats. These results provide evidence that PBPi could protect the brain and testicular tissue against oxidative stress induced by STZ via modulation of serum testosterone concentration and also by enhancement of the antioxidant defence system in STZ-diabetic rats.

CONCURRENT SESSION 02 | CS02
POST-TRANSLATIONAL MODIFICATIONS

CS02.01

CONCURRENT SESSION 02: POST-TRANSLATIONAL MODIFICATIONS
July 18, 2016 10:45 – 12:15

Posttranslational Modification

Brian Raught
Mclaughlin Centre For Molecular Medicine, Ontario Cancer Institute, Toronto, ON, Canada

Abstract: Posttranslational Modification

CS02.02

CONCURRENT SESSION 02: POST-TRANSLATIONAL MODIFICATIONS
July 18, 2016 10:45 – 12:15

Protein Glycation and Its Prevention in Disease and Aging

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University of Rzeszow, Department Biotechnology and Microbiology, Department Biochemistry and Cell Biology, Rzeszów, Poland

Abstract: Protein glycation is one of the main factors contributing to aging and is an important element of etiopathology of age-related diseases, especially type 2 diabetes mellitus. Counteracting glycation and its effects can therefore be a means of increasing both the lifespan and healthspan. The level of protein glycation *in vivo* is a resultant of the rate of glycation and the rate of removal of glycation products. We have studied prevention of protein glycation by various natural and synthetic antioxidants confirming the high efficiency of polyphenols including flavonoids and phenolic acids in protection against glycation induced by monosugars and aldehydes, and identified new efficient protectors. We identified nitroxides, non-toxic synthetic antioxidants of modifiable structure as promising new anti-glycating agents. We studied protein glycation by ascorbate and its prevention by metabolic reduction of ascorbate oxidation products. Our data point to the possibility of participation of the ascorbyl radical in protein glycation. Protein cross-links caused by Advanced Glycation Endproducts (AGEs) can be eliminated by AGE breakers. As many modified proteins are degraded preferentially by proteasomes, activation of proteasomes and stimulation of their biosynthesis is another strategy to decrease the level of intracellular protein glycation. Considerable part of effects induced by protein glycation is due to interaction of AGEs with their receptors (RAGEs). Prevention of RAGE activation by natural and synthetic compounds that compete with AGEs for RAGEs, decrease expression of RAGEs, increase the level of sRAGEs and interfere with the RAGE-initiated signaling pathways may thus be of prophylactic and therapeutic value.

CS02.03

CONCURRENT SESSION 02: POST-TRANSLATIONAL MODIFICATIONS
July 18, 2016 10:45 – 12:15

Baculovirus-Induced Actin Polymerization is Involved in the Mechanism of Nuclear Import of AcMNPV Nucleocapsid

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Abstract: The transport of macromolecules into the nucleus occurs through the nuclear pore complex (NPC) and requires cellular receptors and the RanGTPase cycle. Most of the viruses that replicate in the nucleus of their host cells exploit this mechanism to enter the nucleus. However, we found that the nucleocapsid from the baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV), one of the largest viruses that replicate in the nucleus of their host cells, crosses the NPC and enters the nucleus intact independently of cellular receptors and the RanGTPase cycle. AcMNPV contains the VP78/83 protein that activates the Arp2/3 complex and induces actin polymerization at one end of the rod-shaped nucleocapsid. Two nuclear import assays, one with semi-permeabilized cells and the second with isolated nuclei, were used to study the nuclear import of AcMNPV nucleocapsid independently of its actin-mediated migration towards the NPC. We found that inhibitors of Arp2/3 blocked nuclear import of AcMNPV nucleocapsids. In addition, nuclear import of nucleocapsids was reconstituted in purified nuclei supplemented with G-actin and Arp2/3 under actin polymerization conditions. Thus, we propose that the AcMNPV nucleocapsid breaches the NPC using the mechanical force of actin polymerization to propel itself through the NPC entering the cell nucleus without the need of cellular receptors. Our findings

represent a novel mechanism of nuclear import and point to a very distinct role of actin-based motility during the baculovirus infection cycle.

CS02.04

CONCURRENT SESSION 02: POST-TRANSLATIONAL MODIFICATIONS
July 18, 2016 10:45 – 12:15

Positive and Negative Control of Protein-Serine/Threonine Kinases by Phosphorylation in the Catalytic Domain T-Loop

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Abstract: The catalytic domains of most eukaryotic protein kinases are highly conserved in their primary structures. Their phosphorylation within the activation T-loop between kinase subdomains VII and VIII is a common mechanism for stimulation of their phosphotransferase activities. We have developed hundreds of antibodies to track the stimulatory phosphosites in the T-loops of most human protein kinases that may serve as markers for their catalytically active forms. However, two highly conserved threonine and tyrosine phosphorylation sites (T207 and Y210 in human extracellular signal-regulated kinase 1 (ERK1)) are frequently found in most protein-Ser/Thr kinases (PS/TK's), but not protein-Tyr kinases. ERK1 is a member of the mitogen-activated protein kinase (MAPK) family and serves as a paradigm for regulation of protein kinases in signalling modules. Our studies demonstrate the functional importance of these phosphosites for inhibition of ERK1 catalytic activity. A T207A mutant of ERK1 exhibited full phosphorylation of T202 and Y204 in the absence of the upstream kinase MEK1, but was inactive towards other substrates. The unphosphorylated Y210 site was also important for proper conformational arrangement of the active site, and a Y210F mutant could not be recognized by MEK1 for phosphorylation of T202 and Y204. We propose that after activation of ERK1 by MEK1, subsequent slower phosphorylation of the flanking sites results in its inhibition. Hyperphosphorylation within the kinase activation T-loop may serve as a general mechanism for PS/TK down-regulation after initial activation by their upstream kinases and may partly explain why, unlike protein-Tyr kinases, PS/TK's rarely occur as mutated oncoproteins.

CONCURRENT SESSION 03 | CS03
EXTRACELLULAR MATRIX AND SIGNALING

CS03.01

CONCURRENT SESSION 03: EXTRACELLULAR MATRIX AND SIGNALING
July 18, 2016 10:45 – 12:15

Identification of LPR-1 as a Key Endocytosis Receptor for β1 Integrin in a Tumor Context

Louis Theret[1], Benoit Langlois[1], Anais Navarre[2], Christine Terryn[3], Cathy Hachet[1], Hervé Emonard[1], Sébastien Almagro[1], Stephane Dedieu[1]
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Abstract: LRP-1 is a large endocytic receptor mediating the clearance of various molecules from the ECM. LRP 1-mediated endocytosis was first associated to anti-tumor properties by carrying the uptake and catabolism of serine proteinases and MMPs. However, despite its ability to limit ECM remodeling through endocytosis, LRP-1 may also coordinate the adhesion-deadhesion balance in malignant cells by regulating the cytoskeleton organization and adhesion structure turnover through the activation of MEK/ERK and inhibition of MKK7/JNK pathways to support cancer invasion. To investigate how LRP-1 could regulate the cell-surface proteome, we characterized the cell-surface receptors involved in carcinoma cell adhesion. Our data revealed that β1 integrin level is significantly increased at the cell surface of FTC-133 thyroid carcinomas upon treatment with RAP, used as LRP-1 antagonist. Immunoprecipitation experiments, taught us that LRP-1 and β1 integrin coexists in the same biomolecular complex and immunofluorescence studies revealed a spatial co-localization between these two partners. Biochemical endocytosis assay, highlighted LRP-1 as a mediator of β1 integrin endocytosis in FTC-133. Furthermore, by using specific markers of early endosomes and lysosomes, we demonstrated that the number of integrin-containing endosomes is decreased by about 40% when LRP-1-mediated endocytosis is abolished. Moreover, our data indicate that LRP-1 is specifically involved in β1 integrin recycling but not in lysosome targeting. Overall, we identified an original molecular way in tumor environment involving LRP-1 as a main regulator of β1 integrin internalization and recycling. Further work is currently in progress to decipher how the interaction between LRP-1 and β1 integrin impact tumor progression.

CS03.02

CONCURRENT SESSION 03: EXTRACELLULAR MATRIX AND SIGNALING
July 18, 2016 10:45 – 12:15

MK5 Regulates Collagen Biosynthesis in Cardiac Fibroblasts and May Modulate Extracellular Matrix Remodelling in the Heart

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Abstract: Fibroblasts represent the principal source of collagen in the heart. Increased interstitial fibrosis is a result of changes in both the deposition and breakdown of intracellular matrix proteins, including collagen. We have shown previously that the ability of chronic pressure overload to increase cardiac ventricular Col1α1 mRNA levels and collagen deposition was reduced in MAP kinase-activated protein kinase-5 haploinsufficient ($MK5^{+/-}$) mice. The present study was to determine the mechanisms whereby MK5 regulates fibrosis. Cardiac fibroblasts were isolated from male $MK5^{+/+}$, $MK5^{+/-}$, and $MK5^{-/-}$ mice: Subconfluent cultures from passage 2 were used. MK5 mRNA and immunoreactivity were detected in fibroblasts from $MK5^{+/+}$ mice. mRNA for proteins implicated in extracellular matrix (ECM) remodelling were quantified, with or without treatment with angiotensin II (Ang-II), using pathway-targeted qPCR microarrays. Collagen secretion was assessed by immunoblotting. The profile of ECM mRNAs differed depending on genotype. $MK5^{-/-}$ fibroblasts showed significantly elevated levels of Col1α1, Col1a2, Col13a1, and Timp3 mRNA compared to $MK5^{+/+}$. In contrast, mRNA for MMP13 and MMP8 was reduced. Stimulation with Ang-II (6- and 24-h) had no significant effect on the abundance of these mRNAs. Collagen immunoreactivity was detected in media from unstimulated $MK5^{-/-}$, but not $MK5^{+/+}$, fibroblasts. Ang-II stimulation (24-h) significantly increased the amount of collagen immunoreactivity in media from $MK5^{+/+}$ fibroblasts. In contrast, media from $MK5^{-/-}$ fibroblasts showed increased collagen immunoreactivity after 6-h of Ang-II stimulation with a return to basal levels after 24-h of stimulation. In conclusion, MK5 plays a role in collagen biosynthesis and, possibly, ECM remodelling.

CS03.03

CONCURRENT SESSION 03: EXTRACELLULAR MATRIX AND SIGNALING
July 18, 2016 10:45 – 12:15

The Concept of Ectosteric Inhibitors for the Selective Inhibition of the Degradation of Extracellular Matrix Proteins

Dieter Brömme[1], Xin Du[2], Vidhu Sharma[1], Preety Panwar[1], Simon Law[1]
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Abstract: Cathepsins are potent extracellular matrix protein-degrading proteases exhibiting either elastase and or collagenase activities. The collagenase activity of cathepsin K (CatK) is a major anti-resorptive drug target in skeletal diseases, whereas the elastase activities are considered detrimental in cardiovascular, respiratory, and potentially skin diseases. Substantial efforts have been invested to design highly potent and selective active site-directed CatK inhibitors for the treatment of osteoporosis which all showed efficacy in preclinical and/or clinical studies but were plagued with serious side effects. These side effects may partially be to the inhibition of non-matrix protein degradation functions of the drug target cathepsin. We have identified specific ectosteric sites associated with matrix protein degradation in cathepsins. These sites are needed for the binding to substrates such as elastin and collagen, for the binding of ligands or for protease oligomerization. In contrast to allosteric sites, they do not affect the active site. For example, CatK requires glycosaminoglycans (GAGs) as ligand for the formation of oligomeric protease complexes. Only these complexes are collagenolytically active whereas the monomer exhibits all other proteolytic functions. Preventing oligomerization, GAG binding or non-active site-based interactions with matrix proteins allows for the selective inhibition of the therapeutically relevant elastase and collagenase activities of cathepsins without interfering with their other functions. These inhibitors represent a new class of drugs which specifically block only the disease-relevant activity of a target enzyme and thus avoid potential side effects intrinsic to a multifunctional drug target.

CS03.04

CONCURRENT SESSION 03: EXTRACELLULAR MATRIX AND SIGNALING
July 18, 2016 10:45 – 12:15

Endothelial-Derived Extracellular Matrix Preserves the Stemness of Bone Marrow-Derived Mesenchymal Stem Cells

Ming-Kang Lee[1], Wei-Chun Huangfu[2], I-Hsuan Liu[1]
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Abstract: Mesenchymal stem cells (MSCs) are with great potential in regenerative medicine. However, the physiological nature of MSCs including the endogenous stem cell niche remains elusive. Emerging hypotheses suggested that pericytes residing subendothelium might be one of the primitive origins of MSCs,

and accordingly we speculated that endothelial cells (ECs) might participate in the constitution of the stem cell niche for MSCs. In this study, ECs derivatives including extracellular matrix (EC-ECM) and paracrine factors from conditioned medium (EC-CM) were investigated for the potential to maintain MSCs stemness. When compared with MSCs cultured alone, on MSC-ECM and in EC-CM, MSCs on EC-ECM possessed the morphology of more juvenile cells, showed quiescence in proliferation, and, once induced, resulted in better osteogenic differentiation in concert with the higher expressions of osteogenic genes. These results indicated that EC-ECM could preserve MSC stemness. To further investigate the underlying mechanisms, epigenetic regulatory profile was characterized. We found that MSCs expanded on EC-ECM had significantly higher H3K27me3 with significantly lower KDM6B expression. Taken together, EC-ECM can retain MSCs stemness by shaping an inhibitory chromatin signature via maintaining lower expression of KDM6B. Our work provided supportive evidence that MSCs can reside in a perivascular niche, but the detailed signal between extracellular environment and intracellular chromatin signature requires further investigation.

CONCURRENT SESSION 04 | CS04
RAPID FIRE PRESENTATIONS - CELLULAR REGULATION I

CS04.01

CONCURRENT SESSION 04: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION I
July 18, 2016 10:45 – 12:15

Differential Response of Two Putative Wnt/β-Catenin Target Genes, cx43 and dax1 in 42GPA9 (Mouse Adult Sertoli) Cell Line

Camila Lopez[1], Rodrigo Aguilar[2], Martin Montecino[2], Michael Meisterernst[3], Juan C. Slebe[1], Ilona I. Concha[1]
[1]Instituto De Bioquimica Y Microbiologia, Universidad Austral de Chile, Valdivia, Chile; [2]Laboratorio Regulacion Genica, Universidad Andres Bello, Santiago, Chile; [3]Institute for Molecular Tumor Biology, University of Muenster, Muenster, Germany

Abstract: Sertoli cells are the nutritional and metabolic support of germ cells. Wnt/β-catenin signaling is important for the development of the seminiferous epithelium during embryonic age, however after birth this pathway is downregulated. Transgenic mice where β-catenin is constantly activated have altered spermatogenesis. Cx43 and Dax1 are important proteins for testicular development. These genes have TBEs (TCF binding elements) within their promoters and in transgenic mouse models, cx43 and dax1 are deregulated possibly affecting Sertoli cell functionality. We evaluated whether this signalling pathway induces upregulation of *cx43* and *dax1* gene expression in 42GPA9 cells and the possible molecular mechanism involved in the differential response of these genes. Nuclear translocation of b-catenin was evaluated by immunodetection. mRNA abundance was determined by RT-qPCR and histone marks and b-catenin promoter occupancy at the reported TBEs and two additional TBEs found in *cx43* gene was assessed by ChIP analysis. Luciferase assays in an heterologous system (HEK293 cells) was used to study *cx43* as a direct target. Sertoli cells responded to treatments, accumulating b-catenin within the nucleus and activating *axin2* transcription. Stimulated 42GPA9 cells showed a 2-fold increase of *cx43* mRNA and a 2-fold increase of luciferase units in the heterologous system, while *dax1* mRNA was not affected. Histone marks of activation such as H3K9Ac and H3K4me3 were found only in *cx43* TBE although b-catenin was recruited in both *cx43* and dax1 TBEs. These findings suggest that *cx43* gene is a direct target of b-catenin upon activation of this signaling pathway in 42GPA9 cells. FONDECYT 1141033(JCS)

CS04.02

CONCURRENT SESSION 04: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION I
July 18, 2016 10:45 – 12:15

Epigenetic Activation of LY6K Predicts the Presence of Metastasis in Breast Carcinoma

Jong Hoon Park, Hyun Kyung Kong, Ye Sol Kim, Sonda Som
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Abstract: The role of lymphocyte antigen 6 complex, locus K (LY6K) in breast cancer and as a clinical marker for the prognosis of various cancer has been studied, whereas the epigenetic control of LY6K transcription is not fully understood. Here, we report that breast cancer patients with increased LY6K expression had significantly shorter disease-free and overall survival than the patients with low levels of LY6K by multivariate analysis. LY6K also was upregulated in breast carcinoma tissues from patients with distant metastases than those without distant metastases, downregulating E-cadherin expression. Furthermore, xenograft tumor volumes from LY6K knockdown nude mice were significantly reduced than those of mice treated with control lentivirus. Interestingly, LY6K has a CpG island (CGI) around the transcription start site and non-CGI in its promoter, called a CGI shore. LY6K expression was inversely correlated with methylation in not only CGI but CGI shore, which in turn are associated with histone modifications. Additionally, LY6K methylation was regulated by the PAX3 transcription factor due to the SNP242 mutation in LY6K CGI shore. Taken together, breast cancer risk and metastasis were significantly associated with not only LY6K expression, but also methylation of CGI shore which regulated by SNP242 status. Our results suggest that an understanding epigenetic mechanism of the LY6K gene may be useful to diagnose carcinogenic risk and predict outcomes of patients with metastatic breast cancer.

CS04.04

CONCURRENT SESSION 04: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION I
July 18, 2016 10:45 – 12:15

EGF/Ras/Erk Signalling Controls Growth Through Regulation of tRNA and rRNA Synthesis

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Abstract: Ras signalling promotes growth and proliferation in many tissues throughout animal development. An important challenge is to identify how the Ras-ERK pathway alters cellular metabolism to drive growth. Here we report on the control of both rRNA and tRNA synthesis as growth effectors of EGF/Ras/Erk signalling in *Drosophila*. I find that overexpression of oncogenic Ras (RasV12) leads to increased mRNA translation and protein content in S2 cells, suggesting that Ras may promote growth through enhanced protein synthesis. Next, we investigated how these effects involve control of both rRNA and tRNA synthesis. My data suggest that overexpression of RasV12 or the activated versions of EGFR and the Raf1 in wing imaginal discs increases both rRNA and tRNA synthesis. Similarly, expression of RasV12 in S2 cells increases tRNA and rRNA levels, while blocking Ras/ERK signaling using the MEK inhibitor, U0126 or RNAi reduces rRNA and tRNA synthesis. We previously identified the RNA polymerase I and RNA polymerase III (Pol I and Pol III) factors, TIF-IA and Brf1, as regulators of cell and tissue growth in *Drosophila*. Here we show that knockdown of either TIF-IA or Brf1 blocks the effects of Ras signalling on growth and proliferation in larval wing imaginal discs, adult midgut progenitor cells and adult intestinal stem cells. Several transcription factors have been shown to link Ras signalling to changes in mRNA expression and growth. We propose that transcription of both rRNA and tRNA represent additional effectors of Ras signalling in the control of protein synthesis and growth.

CS04.05

CONCURRENT SESSION 04: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION I
July 18, 2016 10:45 – 12:15

Metabolic Engineering of Photosynthetic Cyanobacterium Synechocystis sp. PCC 6803 for Overproduction of GABA

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Abstract: GABA (γ-aminobutyric acid) is a four carbon non-protein amino acid produced by the decarboxylation of glutamate which is also known as GAD pathway. GABA production is ubiquities in all life forms ranging from prokaryotes to eukaryotes. It functions as an inhibitory neurotransmitter in mammalian brain, whereas it is known to have various environmental stress relieving roles in plants and bacteria. Because of the beneficial functions of GABA and increasing commercial demand, various attempts have been made for chemical and biological synthesis of GABA. However biological synthesis of GABA is considered as a more promising method due to the simple catalytic reaction, cost effectiveness and environmental compatibility. We have engineered the unicellular cyanobacterium *Synechocystis* sp. PCC 6803 (hereafter *Synechocystis*) for enhanced GABA production. An overexpressing vector was used in *Synechocystis* to create stable lines expressing chromosomally integrated *gad*, the gene encoding glutamate decarboxylase in *Synechocystis*. The engineered strain, GAD_{ox}, had a 5-fold increase of GABA content as compared to wild type strain. In another attempt, ketoglutarate decarboxylase, an enzyme that can competitively utilize glutamate in cyanobacteria, was disrupted to create a Δ*kgd* mutant by gene knock-out technique. GABA production was increased by 2- folds in Δ*kgd* mutant strain. It is anticipated that by combining GAD_{ox} and Δ*kgd* mutants to create a double mutant strain, GABA yield could be improved further in *Synechocystis*. This work forms the basis for further development of GABA production by employing cyanobacteria that are safe and eco-friendly microorganisms.

CONCURRENT SESSION 05 | CS05
NEURODEGENERATIVE DISEASE

CS05.01

CONCURRENT SESSION 05: NEURODEGENERATIVE DISEASE
July 18, 2016 13:45 – 15:15

Molecular Bases of the Metabolic Programs of Neurons and Astrocytes

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Abstract: Energy and redox conservation in the brain requires metabolic cooperation between distinct cell types. We have identified mechanisms and factors that maintain cell-specific metabolic programs to allow this metabolic collaboration. Neurons show a high dependence on mitochondrial oxidative metabolism for survival, whereas astrocytes resist to almost complete inhibition of mitochondrial respiration. This is due to the up-regulation, in astrocytes but not in neurons, of glycolysis that generates ATP to maintain the mitochondrial membrane potential reverse ATPase activity. A key metabolic step in this process is PFKFB3 (6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3), an enzyme that promotes glycolysis by activating its regulatory enzyme PFK1 (6-phosphofructo-1-kinase). We showed that PFKFB3 is a substrate of the E3 ubiquitine ligase, anaphase-promoting complex/cyclosome (APC/C)-Cdh1. By regulating PFKFB3 protein stability, APC/C-Cdh1 activity controls the metabolic program, redox status, and survival of neurons and astrocytes.

CS05.02

CONCURRENT SESSION 05: NEURODEGENERATIVE DISEASE
July 18, 2016 13:45 – 15:15

Improved Mitochondrial Function in Huntington's Disease Through Regulation of PDH Activity

Ana Cristina Rego
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Abstract: Reduced mitochondrial function and bioenergetics, including pyruvate dehydrogenase (PDH) dysfunction, have been described in Huntington's disease (HD), an autosomal neurodegenerative disorder linked to polyglutamine expansion at the N-terminal of the huntingtin protein (HTT). We previously showed that histone deacetylase inhibitors (HDACis), trichostatin A and sodium butyrate (SB), ameliorated mitochondrial function in cells expressing full-length mutant HTT (FL-mHTT). In this study we investigate the effect of HDACis on the regulation of PDH activity in striatal cells derived from HD knock-in mice and YAC128 mice brain. Mutant cells exhibited decreased PDH activity and E1alpha subunit protein levels and increased PDH E1alpha phosphorylation/inactivation, accompanied by enhanced protein levels of PDH kinase (PDK)1/3. Treatment with SB and phenylbutyrate, another HDACi, increased histone H3 acetylation, recovered cell viability, mitochondrial respiration and levels of adenine nucleotides in mutant cells. Exposure to SB also decreased PDH inactivation in mutant cells, which was associated with decreased mRNA expression levels of the two most abundant PDK isoforms, PDK2 and PDK3. In accordance, PDK3 knockdown improved mitochondrial function, suggesting that the positive effects achieved with SB treatment may be dependent on PDK3 inactivation. YAC128 mice brain cortex presented higher mRNA levels of PDK1-3 and PDH phosphorylation, as well as decreased energy levels, which were significantly ameliorated following SB treatment. These results suggest that HDACis, particularly SB, may promote the expression and activity of PDH in HD brain, helping to counteract HD-related deficits in mitochondrial bioenergetics.

CS05.03

CONCURRENT SESSION 05: NEURODEGENERATIVE DISEASE
July 18, 2016 13:45 – 15:15

Link Between Pentose Phosphate Pathway and Telomerase Activity in Glioblastoma

Ellora Sen[1], Fahim Ahmad[1], Deobrat Dixit[1], Vikas Kumar[2], Anupam Kumar[2], Shanker Joshi[1], Chitra Sarkar[2]
[1]National Brain Research Centre, Manesar, India; [2]All India Institute of Medical Sciences, New Delhi, India

Abstract: Increased telomerase activity is essential for cancer cell survival, as maintenance of telomere length confers resistance to replicative senescence and supports proliferative potential. TERT promoter mutations that lead to enhanced expression of telomerase occur in several cancers including Glioblastoma multiforme (GBM). In GBM patients, tumor bearing TERT promoter mutations (C228T and C250T) are associated with increased telomerase activity. As aberrant metabolism is an indispensable participant in GBM progression, the role of TERT on glioma cell metabolism was investigated. Pharmacological inhibition of hTERT by Costunolide induced glioma cell apoptosis in a ROS dependent manner. Inhibition of hTERT either by Costunolide, or siRNA or dominant negative hTERT (DN-hTERT) abrogated (i) expression of G6PD and transketolase (TKT)- two major nodes in the pentose phosphate (PPP) pathway; and (ii) phosphorylation of glycogen synthase (GS). hTERT knock-down decreased TKT activity and increased glycogen accumulation. Interestingly, siRNA mediated knock-down of TKT elevated glycogen accumulation. On extending these *in vitro* findings to heterotypic xenograft glioma mouse model, Costunolide was found to reduce tumor burden *in vivo*. Costunolide treated tumors exhibited diminished TKT activity, heightened glycogen accumulation and increased senescence. Importantly, GBM patient tumors bearing TERT promoter mutations (C228T and C250T) exhibited elevated TKT expression and decreased glycogen accumulation. Taken together, our findings highlight the unknown link between telomerase and dysregulated metabolism in GBM.

CONCURRENT SESSION 06 | CS06
EPIGENETIC SIGNALING & REGULATION

CS06.01

CONCURRENT SESSION 06: EPIGENETIC SIGNALING & REGULATION
July 18, 2016 13:45 – 15:15

Nucleosomal Response Pathway and Immediate Early Gene Expression

James R. Davie, Dilshad Khan, Shanan Healy, Shihua He, Carolina Gonzalez, Kiran L. Sharma, Varonica Lau, Tarek Bader, Ifeoluwa E. Adewumi, Wayne Xu
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Abstract: Signaling cascades regulate multiple processes in the cell, including the expression of immediate-early genes (IEGs) in response to different stimuli. Mitogen- and stress-activated kinase 1/2 (MSK1/2), acting downstream of the MAPK signaling pathway, are involved in the regulation of IEGs. In mammalian cells, stimulation of RAS-MAPK-MSK1/2 by EGF, TPA or TNF alpha results in the phosphorylation of H3 at S10 and S28 (the nucleosomal response) and remodeling of the IEG regulatory regions. ChIP assays of human and mouse cells show the transient increase of H3S10ph or H3S28ph at the regulatory regions of IEGs. There is also a transient increase and decrease of H3K4me3 in the IEG coding region. To identify IEGs regulated by the MSK, we have completed transcriptome analyses of mouse and human cell lines stimulated with TPA, EGF and TNF alpha 4 H89, a potent MSK inhibitor. Imaging and sequential ChIP assays demonstrate that an IEG epi-allele is phosphorylated at H3S10 or H3S28, but not at both sites. The level of H3S28ph is responsive to inhibition of histone

deacetylase and CBP. H3S28ph, but not H3S10ph, is associated with H3K27ac, providing evidence that H3K27ac guides MSK to phosphorylate H3 at S28. The recent results of our studies on the MSK-mediated nucleosomal response and gene programming will be presented. (This work was supported by grants from Research Manitoba, CancerCare Manitoba, Canadian Breast Cancer Foundation, and a Canada Research Chair to J.R.D.)

CS06.02

CONCURRENT SESSION 06: EPIGENETIC SIGNALING & REGULATION
July 18, 2016 13:45 – 15:15

The Chromatin Architecture Reveals Functional Noncoding Genetic Alterations in Breast Cancer

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Abstract: Approximately 24,000 new cases of breast cancer per year are estimated in Canada, representing approximately 26% of newly diagnosed cancer cases in the country. Over 5,000 women will die from breast cancer within the same year. Over two thirds of cases rely on oestrogen-dependent activation of the estrogen receptor alpha (ESR1), a potent transcription factor setting an oncogenic expression program in breast cancer cells. Genome-Wide Association Studies (GWAS) and Whole-Genome Sequencing (WGS) have identified both inherited and somatic mutations in ESR1-positive breast cancer. Most of these genetic alterations map to outside of coding sequences. Here we explore the role of these genetic alterations on regulatory elements identified through epigenetic annotation across the genome of ESR1-positive breast cancer cells.

CS06.03

CONCURRENT SESSION 06: EPIGENETIC SIGNALING & REGULATION
July 18, 2016 13:45 – 15:15

Renal Proximal Tubule Na,K-ATPase Is Controlled by CREB-Regulated Transcriptional Coactivators and Salt-Inducible Kinase

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Abstract: The kidney plays a central role in blood pressure regulation, via its ability to control sodium reabsorption via locally produced natriuretic, and anti-natriuretic factors. Previously, natriuretic and anti-natriuretic factors, including dopamine and norepinephrine, have been observed to alter sodium reabsorption by changing the phosphorylation state of Na,K-ATPase in the renal proximal tubule (RPT). Protein Kinase A and calcium-mediated signaling pathways are involved. These same signaling pathways control transcription of the Na,K-ATPase β subunit gene *atp1b1* in RPT cells. In this report, evidence is presented that (1) recently discovered cAMP-regulated transcriptional coactivators (CRTCs), and salt-inducible kinase 1 (SIK1) contribute to the transcriptional regulation of *atp1b1* in RPT cells and (2) renal effectors, including norepinephrine, dopamine, prostaglandins, and sodium, play a role. Evidence for the role of CRTCs includes the loss of transcriptional regulation of *atp1b1* by a dominant-negative CRTC, and a CREB mutant with an altered CRTC binding site. In a number of experimental systems, SIK1 phosphorylates CRTCs, which are then sequestered in the cytoplasm, preventing their nuclear effects. Consistent with such a role of SIK1 in primary RPT cells, *atp1b1* transcription increased in the presence of a dominant-negative SIK1. Regulation by dopamine, norepinephrine, and monensin was also disrupted by a dominant-negative SIK1. These latter observations can be explained if these renal effectors phosphorylate and inactivate SIK1. Our results support the hypothesis that Na,K-ATPase in the RPT is regulated at the transcriptional level via SIK1 and CRTCs, in addition to the previously reported control of the phosphorylation of Na,K-ATPase.

CS06.04

CONCURRENT SESSION 06: EPIGENETIC SIGNALING & REGULATION
July 18, 2016 13:45 – 15:15

RNA Polymerase II Stalling Targets Histone Acetylation to Active Genes

Leann Howe
University of British Columbia, Vancouver, BC, Canada

Abstract: Histone acetylation is a ubiquitous hallmark of transcriptional activity, but whether the link is of a causal or consequential nature is still a matter of debate. By mapping genome-wide histone acetylation levels in *S. cerevisiae*, we reveal that the majority of histone acetylation is dependent on RNA polymerase II (RNAPII) activity and predominantly observed in regions with increased RNAPII stalling. Moreover, consistent with a role of nucleosomes in obstructing progression of RNAPII, previously published algorithms that predict nucleosome stability based on sequence alone can be used to predict histone acetylation. Finally, we show that genetically inducing RNAPII stalling via overexpression of a dominant negative TFIIS mutant promotes histone acetylation. These findings redefine the role of acetylation in transcription, suggesting that it is primarily a response to, and potentially involved in bypass of, nucleosome-induced transcriptional stalling.

CONCURRENT SESSION 07 | CS07
NOVEL THERAPEUTICS

CS07.01

CONCURRENT SESSION 07: NOVEL THERAPEUTICS
July 18, 2016 13:45 – 15:15

Two Hits are Better Than One: Targeting the Metabolic Mevalonate Pathway to Effectively Trigger Tumour Cell Death

Joseph Longo[1], Peter J. Mullen[1], Rosemary Yu[1], Jenna Van Leeuwen[1], Linda Z. Penn[2]
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Abstract: The statin family of drugs are routinely prescribed for the treatment of hypercholesterolemia, but have also been shown to possess pleiotropic anti-cancer properties. We, and others, have shown that statins can induce tumour-specific apoptosis by inhibiting the mevalonate pathway, a crucial metabolic pathway responsible for the production of cholesterol and non-sterol end-products. Our working model is that cancer cells are dependent upon the mevalonate pathway for growth and survival, which is a tumour vulnerability that can be targeted by statins. Indeed, this is consistent with our previous results showing deregulation of the mevalonate pathway can contribute to tumorigenesis.

At a molecular level, cellular exposure to statins inhibits the metabolic mevalonate pathway and triggers a homeostatic feedback response governed by the sterol regulatory element-binding protein (SREBP) family of transcription factors. Recent data from our lab shows that inhibiting this restorative feedback mechanism via SREBP knockdown potentiates statin induced apoptosis. In line with these results, we recently identified another approved agent, dipyridamole, as an inhibitor of SREBP and synergistic potentiator of statin-triggered tumour cell apoptosis both *in vitro* and *in vivo*. This is a new activity for an established agent used for secondary stroke prevention. We are currently employing multiple genome-wide analyses to elucidate the mechanism by which SREBP activation confers resistance to statin-induced apoptosis. These studies will offer the opportunity to better understand the role of SREBP in cancer and potentially identify novel biomarkers of statin response.

CS07.02

CONCURRENT SESSION 07: NOVEL THERAPEUTICS
July 18, 2016 13:45 – 15:15

Structure and Chemical Biology Approaches to Understand Type IV Secretion System Function and Inhibition

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Abstract: Type IV secretion systems (T4SS) are membrane-associated multiprotein complexes that mediate the translocation of macromolecules (proteins, DNA or DNA-protein complexes) across the bacterial cell envelope. They are required for the virulence of many bacterial pathogens and for the transfer of antibiotic resistance genes between bacteria. They constitute interesting targets for the development of anti-virulence drugs and of inhibitors of plasmid conjugation. We study the mechanism of T4SS using the plant pathogen *Agrobacterium tumefaciens* and the mammalian pathogens *Brucella* and *Helicobacter pylori* as well as the plasmid pKM101 conjugation system as models. The results of our work using protein biochemical, structural biological (X-ray crystallography, NMR spectroscopy) and chemical biology approaches will be presented. Based on crystal structures of essential T4SS components (VirB8-like proteins from *Brucella*, pKM101 and *H. pylori*) we identified residues that are essential for dimerization and for interactions of these proteins using *in vivo* as well as *in vitro* assays. The dimerization of VirB8-like proteins was identified as important for their function and we used NMR spectroscopy to identify dimerization-related conformational changes. Next, we used *in vivo* as well as *in vitro* and *in silico* assays to identify inhibitors of protein function and to characterize their binding site(s). Our results demonstrate that small organic molecules can be used as probes for the analysis of the mechanism of T4SS. They may serve as leads for the development of drugs that inhibit the function(s) of T4SS in bacterial virulence and in antibiotic resistance gene transfer.

CS07.03

CONCURRENT SESSION 07: NOVEL THERAPEUTICS
July 18, 2016 13:45 – 15:15

The Efficacy of Topical All Trans Retinoic Acid (Vitamin A) as a Treatment for Melanoma

Ingrid Elisia, Rachel Cederberg, Vivian Lam, Elyse Hofs, Gerald Krystal
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Abstract: We have identified all-trans retinoic acid (ATRA) as a potent immunomodulator in a human blood assay, where whole human blood is challenged *ex-vivo* with *E. coli* to stimulate an inflammatory response. ATRA effectively suppressed a number of *E-coli*-induced pro-inflammatory cytokines and this corresponded with its suppression of a number of signalling pathways, including the JNK pathway in monocytes. ATRA also potently suppressed the Th1 cytokine IFNγ from peripheral blood mononuclear cells (PBMCs) stimulated with anti-CD3 + anti-CD28 without inducing cytotoxicity, showing it is also capable of modulating adaptive immune responses. ATRA also concentration-dependently promoted the skewing of murine macrophages to an M2 phenotype, as indicated by enhanced expression of Arg-1, in IL-4-stimulated macrophages, further confirming its anti-inflammatory potential. In addition to its immunosuppressive properties, ATRA is also capable of inducing the differentiation of some cancer cells, with acute promyelocytic leukemic (APL) blasts being the best known example. To test its effects on a solid tumor, we tested *in*

vitro, if it could induce the differentiation of B16-F10 melanoma cells, as assessed by increased melanin production, and found that, when combined with DMSO, it synergistically induced the differentiation of these cells. We thus tested the efficacy of ATRA, administered topically, to C57BL/6 mice implanted with B16-F10 melanoma cells and found that ATRA dissolved in DMSO was significantly more effective in suppressing the growth of B16-F10-implanted tumour in C57Bl/6 mice than DMSO- or water-treated groups. We conclude that the use of topical ATRA for the treatment of melanoma warrants further investigation.

CS07.04

CONCURRENT SESSION 07: NOVEL THERAPEUTICS
July 18, 2016 13:45 – 15:15

The Novel Antimicrobial Peptides Pa-MAP2 and 1.9 Have Different Mechanisms of Action against Bacterial Infections

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Abstract: During the last decades, the incidence of pathogens multi-resistant to conventional antibiotics has increased, mostly due to the antibiotic over dosage that tends to be applied in healthcare. Thus, it is critical to find new alternatives to overcome the limitations that therapeutics faces nowadays. A possible alternative is given by antimicrobial peptides (AMPs), short amphipathic cationic peptides, with broad-spectrum activity against different types of pathogens (bacteria, fungi and even cancer cells), with low propensity to resistance development. The mechanisms that are responsible for AMPs action are not well understood, being mostly associated to the initial membrane interaction. This work is focused in two different AMPs (*Pa*-MAP2 and 1.9), synthetically designed from the same precursor, in order to improve their activity. Their action against Gram-negative bacteria (using lipid vesicles as biomembrane model systems and *Escherichia coli* cells) was assessed through several methods, including surface plasmon resonance and flow cytometry. Different fluorescence probes allowed us to observe that both AMPs do not interfere with fluidity or membrane lipid packing. On the contrary, membrane surface and dipole potentials were affected by both peptides, although in different ways. Zeta-potential, dynamic light scattering and atomic force microscopy results were in agreement with the previous findings. Other aspects of the AMPs interaction with the target cells are still being addressed, but it is already clear that the way through which the interaction with biomembranes takes place can promote distinct mechanisms of action between these peptides.

CONCURRENT SESSION 08 | CS08
RAPID FIRE PRESENTATIONS - CELLULAR REGULATION II

CS08.01

CONCURRENT SESSION 08: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION II
July 18, 2016 13:45 – 15:15

KRT Interacts with β-Catenin/RAC1 Complex to Regulate NUMB-Dependent NOTCH Signaling Pathway

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Abstract: Studies have reported that interactions between keratins (KRTs) and other proteins initiate signaling cascades that regulate cell migration, invasion, and metastasis. In the current study, we found that expression of keratin (KRT) was specifically high in breast cancers and significantly correlated with their invasiveness. Moreover, knockdown of *KRT* led to increased proliferation, migration, invasion, drug resistance, and sphere formation in breast cancer cells via an upregulated NOTCH signaling pathway. This was due to reduced expression of NUMB, an inhibitory protein of the NOTCH signaling pathway. Additionally, we found that KRT interacts with β-catenin/RAC1 complex and enhances the nuclear translocation of β-catenin. Concordantly, knockdown of *KRT* suppressed the nuclear translocation of β-catenin as well as β-catenin-mediated NUMB expression. Furthermore, modulation of KRT-mediated regulation of NUMB and NOTCH1 expression led to the repression of the cancer stem cell properties of breast cancer-patient-derived $CD133^{high}/CXCR4^{high}/ALDH1^{high}$ cancer stem-like cells (CSLCs) which showed very low *KRT* and high *NOTCH1* expression. Taken together, our study suggests a novel function for KRT in the regulation of nuclear import of the β-catenin/RAC1 complex, thus modulating the NUMB-dependent NOTCH signaling pathway in breast cancers and CSLCs, which might bear potential clinical implications for cancer or CSLC treatment.

CS08.02

CONCURRENT SESSION 08: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION II
July 18, 2016 13:45 – 15:15

EphB6 Supports Expansion of Tumour-Initiating Cells and Suppresses Drug Resistance in Triple Negative Breast Tumours

Behzad Toosi[1], Odette Allonby[2], Matthew Shannon[3], Darrell Mousseau[1], Peter Siegel[4], Deborah Anderson[5], Franco Vizeacoumar[5], Anthony Kusalik[3], Andrew Freywald[2]
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QC, Canada; [5]Saskatchewan Cancer Agency, Saskatoon, SK, Canada

Abstract: Triple negative breast cancer (TNBC) is associated with a high resistance to therapy and early tumour relapse. This is likely due to the resistance of slowly-proliferating tumour-initiating cells (TICs). In our recent work, we found that a catalytically-inactive member of the Eph group of receptor tyrosine kinases, EphB6, partially suppresses the epithelial-mesenchymal transition in TNBC cells, while promoting expansion of triple negative TICs. Its proliferative effect is mediated by the activation of the Erk pathway, predominantly by the Erk2 kinase. In xenograft models, EphB6 accelerates tumour growth and potentiates tumour initiation. Remarkably, EphB6 also suppresses tumour drug resistance, probably by forcing TICs into a more proliferative, drug-sensitive state. In agreement, patients with higher EphB6 expression in TNBC tumours have a better chance for recurrence-free survival. These observations suggest that it may be beneficial to support EphB6 action concurrent with applying conventional therapies, as it would decrease TIC-mediated resistance and reduce recurrence of TNBC**.** These findings are of a high level of general interest, as they not only reveal a new molecular mechanism that governs the behaviour of TICs, but remarkably, they also imply that the enhanced proliferative activity of TICs is not necessarily a bad factor for cancer patients, since it may make cytotoxic treatments more effective by increasing sensitivity of TICs to DNA-damaging agents. This counterintuitive paradigm cautions against inhibiting molecules that support TIC proliferation, since this treatment is likely to drive TICs into a more dormant, drug-resistant state, especially if it is used in combination with DNA-damaging therapies.

CS08.03

CONCURRENT SESSION 08: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION II
July 18, 2016 13:45 – 15:15

Antiperoxidative, Anti-Inflammatory, Cytotoxic and Genotoxic Properties of Morinda Lucida BENTH

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Abstract: Biological peroxidation, inflammation, cytotoxicity and genotoxicity are frequently linked with cancers. With inadequate and poor health systems in Africa, there is currently a huge drift towards use of medicinal plants to manage/treat many debilitating diseases including cancers. One such plant is *Morinda lucida*. Thus, its parts extracts were studied for anti-oxidant, anti-inflammatory, cytotoxic and genotoxic/genoprotective properties. Anti-oxidation assays presented highest SOD activity by the bark. GPx activity was significantly higher in both bark and leaf extracts while CAT activity was highest in leaf ($p<0.05$). Anti-inflammatory properties at doses of 100, 300 and 500mg/kg on carrageenan-induced rat paw oedema were in dose-dependent manner. Respectively, leaf inflammatory inhibition (%) of: 83.53410.50, 91.6748.33, and 100.0040.00; stem bark: 58.33415.37, 91.6748.33 and 91.6748.33; fruit: 58.33415.37, 75.00417.08 and 100.0040.00; and, root: 100.0040.00 at all the doses was recorded. Cytotoxicity examination by brine shrimp (hatchability and lethality) assays revealed potency (IC50) in the order: fruit>root>leaf>stem bark, at 170mg/L against artemia cysts. Lethality assay revealed leaf possessing highest cytotoxicity among all plant parts, at 316.2mg/L followed by root<fruit<stem bark. Genotoxic/genoprotective properties of plant parts on pUC18 plasmid DNA were studied, in presence of Fenton's reagent by plasmid nicking assay. While fruit, bark and root extracts protected pUC18 plasmid DNA at lower doses particularly, at 10×g/×l, leaf was genotoxic at all the doses used, as evidenced by the presence of DNA linear forms. Results clarify *Morinda lucida* parts possessing significant anti-oxidant, anti-inflammatory, cytotoxic and genotoxic properties, to justify their uses in folkloric medicine, and further investigation scientifically.

CS08.04

CONCURRENT SESSION 08: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION II
July 18, 2016 13:45 – 15:15

Generating Mouse Models to Study Mitochondrial DNA Disease and Aging

James B. Stewart
Max Planck Institute for Biology of Ageing, Cologne, Germany

Abstract: Mitochondria contain their own distinct DNA molecules, which in animals, transmit in an explicitly non-Mendelian manner through the female germline. In the soma, they continue their own replication and turn over, independent of the normal cell cycle control of the nuclear DNA. We have known for over 25 years that mutations of the mitochondria's own DNA can lead to human disease. Thought these diseases are rare in the population, 1 in 200 of us carries one of these mutations within our mitochondria, at subclinical levels. Analyses of normal, aged human tissues have revealed evidence of mitochondrial dysfunction in a mosaic pattern of the cells with these samples. Could these sub-clinical, deleterious mutations play a role in our normal ageing process? Research models have long been lacking, as animal mitochondria cannot yet be *transgenically* manipulated. To circumvent this limitation, we use mice expressing a proof-reading deficient mitochondrial DNA polymerase. These female mice transmit mutations to the mitochondria of their offspring, allowing us to identify and segregate mitochondrial mutations into maternal lineages of mice. Combined with an *in vivo* assay on a permissive tissue, we can directly identify mutations causing mitochondrial dysfunction at an early stage in the breeding scheme, to select the lines carrying the pathogenic mitochondrial DNA mutations. These lineages are then utilized as models of both the study of mitochondrial mutations in the ageing process, and to generate mouse lines that can serve as models for basic research and translational studies into mitochondrial DNA-based genetic disorders.

CS08.05

CONCURRENT SESSION 08: RAPID FIRE PRESENTATIONS - CELLULAR REGULATION II
July 18, 2016 13:45 – 15:15

Regulation of Bcl-xL-ATP Synthase Interaction by Mitochondrial Cyclin B1-Cdk1 Determines Neuronal Survival

Miguel Veas-Pérez De Tudela, María Delgado-Esteban, Angeles Almeida
Institute of Biomedical Research of Salamanca, Salamanca, Spain

Abstract: The survival of post-mitotic neurons needs continuous degradation of cyclin B1, a mitotic protein accumulated aberrantly in the damaged brain areas of Alzheimer's disease and stroke patients. Degradation of cyclin B1 takes place in the proteasome after ubiquitylation by the anaphase-promoting complex/cyclosome (APC/C)-Cdh1, an E3 ubiquitin ligase that regulates cell cycle progression and several key neuronal functions including survival and metabolism. We previously described that APC/C-Cdh1 is highly active in neurons. However, during excitotoxic damage -a hallmark of stroke and other neurological disorders- APC/C-Cdh1 is inactivated, causing cyclin B1 stabilization and neuronal death through an unknown mechanism. Here, we show that an excitotoxic stimulus in rat cortical neurons in primary culture promotes aberrant cyclin B1 accumulation in the mitochondria, in which it binds to, and activates, cyclin-dependent kinase-1 (Cdk1). The cyclin B1-Cdk1 complex in the mitochondria phosphorylates the anti-apoptotic protein B-cell lymphoma extra-large (Bcl-xL), leading to its dissociation from the ß subunit of the F1Fo-ATP synthase. The subsequent inhibition of ATP synthase activity causes complex I oxidative damage, mitochondrial inner membrane depolarization, and apoptotic neuronal death. These results unveil a previously unrecognized role for mitochondrial cyclin B1-Cdk1 complex in the oxidative damage associated with neurological disorders. This work was funded by FEDER and Instituto de Salud Carlos III (PI12/0685)

CONCURRENT SESSION 09 | CS09
CANCER CELLS AND MEMBRANE

CS09.01

CONCURRENT SESSION 09: CANCER CELLS AND MEMBRANES
July 19, 2016 10:45 – 12:15

The Many Lives of Antibodies to Gangliosides: Implications in Developing and Improvement of Immunotherapies

Jose L. Daniotti
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Abstract: Differentially expressed tumor-associated carbohydrates represent a general phenomenon observed in many types of cancer cells. Carbohydrates covalently attached to gangliosides, sialic acid-containing glycolipids, are not the exception. Neosynthesized gangliosides observed in oncogenic processes show antigen specificity and, therefore, they are attractive candidates for the design of cancer immunotherapies. In addition, antibodies to gangliosides have been associated with a wide range of neuropathy diseases and differential cell internalization of these antibodies is associated with different clinical outcome of the diseases. According to information obtained from our and other laboratories, antibodies to different gangliosides species do not share common features like membrane binding, cellular endocytosis and immune response. Rather, they behave in an epitope- and cell type-dependent manner. This information has important translational implications for the understanding of clinical characteristics of anti-glycolipid antibody-mediated neuropathies and for the development of innovative therapeutics targeting. In this sense, the disialo ganglioside GD3 has emerged as a novel and attractive class of cell surface molecule (glycolipid) for targeted delivery of drugs. Its accessibility, high expression in many tumor cells, and mainly its capacity to undergo endocytosis after binding with antibodies, has allowed us to develop an ablation cell strategy for selective intracellular delivery of cytotoxic agent to GD3-expressing cells, providing a rationale for future therapeutic intervention in cancer.

CS09.02

CONCURRENT SESSION 09: CANCER CELLS AND MEMBRANES
July 19, 2016 10:45 – 12:15

Caveolin-1, a Jekyll and Hyde in Cancer

Andrew F.G. Quest
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Abstract: Cancer is a leading cause of human suffering and death worldwide. A central dogma in cancer research over the last decades has been that "gain-of-function" in oncogenes or "loss-of-function" in tumor suppressor genes, contribute to the genesis of this disease. Generally, any given protein will belong to one or the other category, but not to both. Paradoxically, Caveolin-1 (CAV1), a member of the caveolin family of scaffolding proteins, is implicated in cancer development and progression both as a tumor suppressor and promoter of metastasis. Research from this laboratory has shed light on this enigma in linking tumor suppression by CAV1 to E-cadherin-dependent suppression of genes, including survivin and cyclooxygenase-2, and augmented apoptosis. Alternatively, in the absence of E-cadherin, enhanced CAV1 tyrosine-14 phosphorylation activates of a novel Rab5-Rac1 signaling axis to promote tumor cell migration, invasion and metastasis. In the presence of E-cadherin, CAV1 tyrosine-14 phosphorylation, signaling via this pathway and metastasis are suppressed. Thus, CAV1 switches roles in an E-cadherin-dependent manner.
Acknowledgements: FONDECYT-FONDAP 15130011, FONDECYT 1130250

CS09.03

CONCURRENT SESSION 09: CANCER CELLS AND MEMBRANES
July 19, 2016 10:45 – 12:15

Sumoylation Pathways Regulating EMT in Mammary Epithelial Cells and Tumor Metastasis

Shirin Bonni
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Abstract: Epithelial-mesenchymal transition (EMT) is a fundamental biological process that regulates tissue development and tumor progression and metastasis. My laboratory has identified sumoylation-dependent mechanisms that control EMT in mammary epithelial cells. In particular, we have discovered that sumoylation of the transcriptional regulator SnoN plays a critical role in suppressing the ability of the cytokine TGFβ to induce EMT in mammary epithelial cells. We have identified the protein PIAS1 as a SUMO E3 ligase for SnoN that suppresses TGFβ-induced EMT (*Netherton and Bonni PLoS One 2010*). Remarkably, the PIAS1-SnoN sumoylation pathway suppresses TGFβ-induced breast cancer invasion and metastasis (*Dadakhujaev et al. Oncosci 2014*).Using computer-assisted interaction proteomics, we recently identified the ubiquitin ligase TIF1γ as a specific interactor of SnoN1 (*Ikeuchi et al. J Biol Chem 2014*). Strikingly, TIF1γ operates as a SUMO ligase, rather than an ubiquitin ligase, for SnoN1. Importantly, the TIF1γ-SnoN1 sumoylation pathway opposes TGFβ-induced EMT and disrupt the morphogenesis of breast acini in 3D-cultures of epithelial cells. Our ongoing research is focusing on the interplay between PIAS1 and TIF1γ in control of SnoN sumoylation, and EMT and tumor invasion and metastasis. We are also investigating how sumoylation regulates the ability of SnoN to control TGFβ-induced EMT. Collectively, our research has identified the transcriptional coregulator SnoN sumoylation as a key mediator of the ability of the SUMO pathway to suppress TGFβ-induced EMT. At the meeting, I will present new unpublished studies on the role of sumoylation pathways in the regulation of EMT in mammary epithelial cells and tumor metastasis.

CS09.04

CONCURRENT SESSION 09: CANCER CELLS AND MEMBRANES
July 19, 2016 10:45 – 12:15

CKAP4 Functions as a Novel Receptor for Dickkopf1, a Wnt Signal Inhibitor, and Might Be a Molecular Target for Cancer Therapy

Akira Kikuchi, Katsumi Fumoto, Hirokazu Kimura
Molecular Biology and Biochemistry, Graduate School of Medicine, Osaka University, Suita, Japan

Abstract: Dickkopf 1 (Dkk1) is a secretory protein and antagonizes oncogenic Wnt signaling by binding to the Wnt coreceptor low-density lipoprotein receptor-related proteins 6 (LRP6). Dkk1 is also suggested to regulate its own signaling to promote cancer cell proliferation, and the anti-Dkk1 antibody suppresses lung cancer cell-induced xenograft tumor formation. However, the underlying mechanism by which Dkk1 promotes cancer cell proliferation has remained to be clarified. Here we identified cytoskeleton-associated protein 4 (CKAP4), which was originally reported as an endoplasmic reticulum-localized membrane protein, as a novel Dkk1 receptor. Dkk1 bound CKAP4 and LRP6 through the different sites, and Dkk1 induced the internalization of cell surface-localized CKAP4 via a clathrin-dependent manner. Dkk1-CKAP4 signaling activated AKT through the formation of a complex between the proline-rich domain of CKAP4 and the Src homology domain 3 (SH3) of phosphatidylinositol 3-kinase (PI3K), resulting in normal and cancer cell proliferation. Both Dkk1 and CKAP4 were frequently expressed in tumor lesions of pancreatic and lung cancers and their simultaneous expression was negatively correlated with prognosis. Knockdown of CKAP4 from cancer cells reduced their xenograft tumor formation in immunodeficient mice. The anti-CKAP4 antibody blocked the binding of Dkk1 to CKAP4, thereby suppressing AKT activity in cancer cells and inhibiting xenograft tumor formation by cancer cells. Thus, CKAP4 might represent a novel therapeutic target for cancers expressing both Dkk1 and CKAP4.

CONCURRENT SESSION 10 | CS10
EPIGENETIC SIGNALING & REGULATION

CS10.01

CONCURRENT SESSION 10: EPIGENETIC SIGNALING & REGULATION
July 19, 2016 10:45 – 12:15

Epigenetic Control of Mesenchymal Cell Fate

Martin Montecino
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Abstract: Multiple dimensions of epigenetic control contribute to regulation of gene expression during mammalian cell differentiation. In this presentation, the evidence presented supports the role of epigenetic mechanisms during regulation of transcription of critical genes for mesenchymal cell lineage commitment. Special attention is dedicated to discuss recent results leading to the identification of key epigenetic mechanisms that contribute to both silencing and activation of bone-master regulators during differentiation of mesenchymal uncommitted cell precursors to non-osteoblastic and osteoblastic cell types, respectively. Our results shed light on how chromatin-remodeling processes can be associated with the deposit and elimination of post-translational modifications in histones H3 and H4 that are bound to the promoter region of phenotypic genes as well as with changes in DNA methylation status that accompany gene activation and repression. Importantly, these data demonstrate that key members of the polycomb and trithorax group of transcriptional regulators as well as proteins mediating DNA methylation and de-methylation, are critical components during transcriptional control of osteoblast-related genes.

CS10.02

CONCURRENT SESSION 10: EPIGENETIC SIGNALING & REGULATION
July 19, 2016 10:45 – 12:15

Protein Complexes that Modify Chromatin for Transcription

Jerry L. Workman
Workman Laboratory, Showers Institute for Medical Research, Kansas City, MO, United States of America

Abstract: Our laboratory purifies and studies the mechanisms of protein complexes that modify chromatin for transcription. We have purified several histone acetyltransferase complexes that function as transcription co-activators. Some of the complexes contain additional histone modifying activities and interact with sequence specific transcription factors and the preinitiation transcription complex. We have found that histone acetylation is closely linked to chromatin remodeling, recruiting and activating chormatin remodeling complexes at active genes. More recently we have studied histone methyltransferase that function during transcription elongation. Histone methylation during elongation suppresses the occurance of antisense transcripts within open reading frames by directing nucleosome-spacing activities and histone deacetylases to transcribed genes. Finally we have purified a metabolic enzyme complex that interacts with histone methyltransferases at the promoter of genes to provide S-adenosylmethionine for histone methylation and Acetyl-CoA for histone acetylation, while phosphorylating histone H3.

CS10.03

CONCURRENT SESSION 10: EPIGENETIC SIGNALING & REGULATION
July 19, 2016 10:45 – 12:15

Growth Factor Independence 1b (Gfi1b) Regulates Wnt Signaling in Hematopoietic Cells by Recruiting LSD1 to β-Catenin Targets

Peiman Shooshtarizadeh[1], Anne Helness[1], Hugues Beauchemin[2], Charles Vadnais[2], Riyan Chen[2], Tarik Moroy[2]
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Abstract: Growth factor independent 1b (Gfi1b) regulates gene expression by recruiting histone-modifying enzymes such as deacetylases (HDAC), de-methylases (LSD1) or methyl-transferases (G9a) to target gene promoters. Ablation of Gfi1b in mice causes an expansion of hematopoietic stem cells (HSC) and megakaryocytes (Mk) in bone marrow and peripheral blood. Here we present evidence that Gfi1b modulates the Wnt/beta-catenin signaling pathway. Gfi1b interacts with beta-catenin and several inhibitory members of the canonical Wnt/beta-catenin pathway such as APC, CHD8, CtBP, Pontin 52 and the Groucho protein TLE3. Similar to LSD1, CtBP interacts with the SNAG domain of Gfi1b, but Pontin52, CHD8 and TLE3 bind to a newly identified N-terminal domain in Gfi1b that is conserved in several species. Both Gfi1b deficient HSCs and MKs show de-regulation of expression of many Wnt/beta-catenin target genes and Gfi1b and beta-catenin co-occupy their promoters. Interestingly, Gfi1b requires binding to LSD1 to activate beta-catenin/TCF mediated transcription. We present evidence that Gfi1b recruits LSD1 to beta-catenin containing complexes at target gene promoters enabling H3K9 Lysine demethylation and -acetylation. Finally, activating the Wnt/beta-catenin signaling pathway in Gfi1b deficient HSCs and MKs significantly counteracts the effect of Gfi1b deficiency and reduces the expansion of these cells to normal levels. Our data indicate that Gfi1b is a critical factor controlling the cellularity of HSCs and MKs by regulating the expression of Wnt/beta-catenin target genes through histone modifications.

CS10.04

CONCURRENT SESSION 10: EPIGENETIC SIGNALING & REGULATION
July 19, 2016 10:45 – 12:15

The Lysine Acetyltransferase Complex NuA4 Regulates Cellular Phosphatidylinositol-4-Phospahate and Phospholipid Metabolism

Louis Dacquay, Michael Kennedy, Kristin Baetz
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Abstract: Dysregulation of phosphatidylinositol-4-phospahate (PI-4-P) lipid metabolism is associated with many diseases, including neurological diseases such as Alzheimer's; therefore, it is critical to understand how cells regulate PI-4-P homeostasis. In *Saccharomyces cerevisiae*, Golgi-localized PI-4-P levels are tightly regulated by conserved lipid-binding proteins with antagonistic functions. Sec14 activates the PI-kinase, Pik1, to promote synthesis of Golgi-localized PI-4-P, while Osh4 activates the PI-4-P phosphatase, Sac1, to promote depletion of Golgi-localized PI-4-P. It remains poorly understood how these proteins are regulated or if other pathways contribute to modulating PI-4-P metabolism upon changes in steady-state levels of this lipid. We have determined that the post translational modification lysine acetylation is contributing to the cellular response to PI-4-P depletion. Through a systematic analysis of all Lysine Acetyltransferases (KATs) deletion mutants, we found that mutants of the NuA4 KAT complex are hypersensitive to *SEC14* inactivation and *OSH4* overexpression, two conditions where Golgi PI-4-P levels are depleted. Through transcriptome profiling we determined that in the absence of Sec14 function NuA4 regulates the transcription of the CDP-diglyceride synthetase gene, *CDS1*, which produces CDP-DAG- the main lipid precursor for PI-4-P and other phospholipid biosynthesis. Genetic analysis and microscopy analysis confirms that NuA4-dependent regulation of *CDS1* is essential to compensate for defects in Sec14 and decreases in PI-4-P. Altogether, we have determined a newly discovered role for NuA4 as a regulator of PI-4-P metabolism and phospholipid in yeast.

CONCURRENT SESSION 11 | CS11
NEW TECHNOLOGIES

CS11.01

CONCURRENT SESSION 11: NEW TECHNOLOGIES
July 19, 2016 10:45 – 12:15

Target and Biomarker Identification Platform to Design New Drugs against Aging and Age-Related Diseases

Peter Fedichev, Ivan Molodtsov
GERO, Moscow, Russian Federation

Abstract: Our scientific team develops omics data analysis methods to identify potential targets for therapeutic intervention against age-related diseases and aging. Since modern omics data is high dimensional, i.e. the number of features in it is much higher than the number of measurements, the use of traditional machine learning methods is impossible due to the emerging problem of overfitting. Therefore, it is necessary to develop new mathematical methods to analyze this type of data. In our work we use the models of statistical physics to analyze gene networks stability and predict their dynamics over time. The proposed models allow us to link gene network stability with mortality. The models we developed were validated on omics data of different types, such as transcriptome, proteome and metabolome measured in different tissues of various organisms. Our techniques open new opportunities to identify targets to develop new therapy candidates against aging.

CS11.02

CONCURRENT SESSION 11: NEW TECHNOLOGIES
July 19, 2016 10:45 – 12:15

Development of Robust in Vitro 3D Models of Human Tumours for the Identification and Evaluation of Anti-Cancer Drugs

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Abstract: Currently in vivo testing of animals is still the gold standard for drug development and testing of cytotoxic compounds. With the demand for non-animal alternatives rising, here we compare the development and functionality of two 3D culture models for in vitro tumour spheroids.3D tumour spheroids were generated from lung cancer cell lines with EGFR mutations, as well as primary isolated lung cancer epithelial cells. Firstly by encapsulation within a synthetic extracellular matrix to produce isogenic cell populations. It was also possible to produce multi cellular tumour spheroids MCTS in the same manner. Additionally spheroids were co-cultured alongside growth arrested primary fibroblasts, both normal and cancer associate and in the presence of extracellular matrix. Following generation (7 days - 8 weeks), spheroids (~500×m diameter) were treated with cytotoxic agents and characterized by confocal microscopy. Secondly 3D tumour spheroids were generated using Asterand bioscience OrganDot™ system. Consistent responses are achieved in OrganDOT™ cultures. The long-term viability of OrganDOT™ cultures enables sequential or chronic compound testing, while the advancements in functionality, robustness, throughput, and study design enabled by the OrganDOT™ model provide relevant human data on the effects of test compounds in a rapid and reproducible manner. It was possible to determine drug efficacy within the various 3D in vitro models of lung cancer tumours, additionally by utilising cell lines with specific EGFR mutations we were able to probe a range of efficacy on different agents examined.

CS11.03

CONCURRENT SESSION 11: NEW TECHNOLOGIES
July 19, 2016 10:45 – 12:15

A Cutting-Edge Surfaceome Approach to Unveil Key Players in Breast Cancer

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IRIC - University of Montreal, Montréal, QC, Canada

Abstract: Despite continuous efforts, breast cancer remains the leading cause of cancer-related death in women. Occurring respectively in 20-25% and 40% of breast tumors, *ERBB2* amplification and *PIK3CA* activating mutations (E545K, H1047R) are among the most common genomic aberrations in human breast cancer. However, significant advances in therapies targeting HER2 and PI3K oncoproteins fail to demonstrate good efficacy, mainly due to tumor heterogeneity and the development of acquired resistance. To expand the treatment options for breast cancer, new targets are desperately needed. In this respect, the characterization of cell surface proteome (surfaceome) changes occurring in transformed cells is essential to identify novel targets for cancer therapy and diagnosis. Insights into the complexity of the surfaceome have been yet limited by the lack of suitable methodologies. Herein, we have optimized a state-of-the-art proteomics approach based on the labeling of cell surface proteins with biotin reagents, their subsequent purification with avidin chromatography, and quantification using label-free quantitative proteomics with liquid chromatography-tandem mass spectrometry (LC-MS/MS). We have employed this proteomics approach to identify secreted and plasma membrane proteins that are differentially expressed on the cell surface of several MCF-10A human mammary epithelial cell lines that reflect the initiation of breast cancer induced either by HER2 overexpression or *PIK3CA* mutations. Interestingly, our LC-MS/MS analyses identified over 200 cell surface proteins in MCF-10A overexpressing HER2 from which 35% were significantly upregulated compared with isogenic MCF-10A cells. This molecular description of the surface of *ERBB2*-transformed cells will allow to characterize new putative targets for breast tumors.

CS11.04

CONCURRENT SESSION 11: NEW TECHNOLOGIES
July 19, 2016 10:45 – 12:15

Getting More From Telomere QFISH: Correlation of Super Resolution Microscopy and X-ray Spectroscopy

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Abstract: The process of cell ageing is thought to be governed in part by the shortening of a region of DNA found on the end of each chromosome; the telomere. There are various ways to measure the length of telomeres in cells, notably QFISH, where a fluorescent probe binds to telomere repeats, and the length is proportional to the light intensity[1]. However, most reports use this as a relative measure of length usually as a ratio to the light intensity from similarly probed centromeric regions[2].

Here, we aim to make QFISH an absolute quantifiable technique with a measurement of base pairs of DNA for each telomere.

To achieve this, we are applying two complementary techniques; Stochastic Optical Reconstruction microscopy (STORM) which utilises "blinking" fluorophores which can be individually counted, and synchrotron X-ray fluorescence spectroscopy (XRF) performed at the Diamond synchrotron light source, as well as other accelarators[3].

We have developed custom telomere peptide nucleic acid probes bound to gold nanoparticles and an alexa647 fluorescent dye we believe are quantifiable using both XRF and STORM.

We analyse telomeres from an ageing population of embryonic human lung cells (passages from "young", "middle aged" and "old age" cells). We present images of telomeres and compare the applicability of STORM and XRF as techniques to measure length.

Being able to measure absolute lengths of individual telomeres could lead to new insights into the precise mechanisms by which cells enter senescence.

CONCURRENT SESSION 12 | CS12
RAPID FIRE PRESENTATIONS - CANCER ORIGINS AND TREATMENT

CS12.01

CONCURRENT SESSION 12: RAPID FIRE PRESENTATIONS - CANCER ORIGINS AND TREATMENT
July 19, 2016 10:45 – 12:15

FGF2 Enhances Replicative Stress and Leads to Permanent DDR and G2 Cell Cycle Block Selectively in K-Ras Transformed Cells

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Abstract: Introduction We have previously reported that FGF2, despite of being a bona fide growth-factor, irreversibly inhibits proliferation in Ras-dependent malignant mouse cells, but not in immortalized non-tumorigenic cell-lines. Its mechanism is still unclear. Here we report that FGF2 increases basal levels of replicative-stress in Ras-overexpressing Y1 tumor cells, leading to permanent higher levels of activated DNA-damage-response and persistent cell-cycle block in G2-phase. Objective To uncover the mechanism of FGF2-citotoxicity in Ras-transformed mouse cells, taking the Y1 tumor cell-line as model. Results and Discussion FGF2 prevented mitosis initiation in Y1 cells, rendering an irreversible cell-cycle block in G2-phase. Phosphorylated levels of the ATR-ChK1-Wee pathway were higher in FGF2-treated cells; and caffeine, an ATR inhibitor, prevented G2 cell accumulation. These results suggest that the ATR-ChK1-Wee axis is the responsible for FGF2-induced cell cycle block in Y1 cells. As the ATR-ChK1-Wee pathway is activated in response to replicative-stress and DNA-damage, we next accessed γH2AX levels. It revealed that Y1 cells present a constitutive basal level of replicative-stress, which might be consequence of its constitutive K-Ras high-expression state. FGF2 largely increased γH2AX levels, and about 90% of γH2AX positive cells were in S/G2-phases, indicating that FGF2-induced H2AX phosphorylation results from increased DNA replication stress. Conclusions In a context of high basal levels of K-Ras-GTP, FGF2 exacerbated basal proliferative-stress, resulting in permanent activation of DNA-damage signaling and irreversible arrest in G2-phase. We will next focus on the dynamics of the replication fork under FGF2 treatment and the destination of cells after long times of G2-blockage.

CS12.02

CONCURRENT SESSION 12: RAPID FIRE PRESENTATIONS - CANCER ORIGINS AND TREATMENT
July 19, 2016 10:45 – 12:15

Assembly and Functions of Mitochondrial Respiratory Complex II in Breast Cancer

Ayenachew Bezawork-Geleta[1], Bing Yan[1], Lanfeng Dong[1], Dana Pascovici[2], Xiaomin Song[2], Mark P. Molloy[2], Jiri Neuzil[1]
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Abstract: Mitochondrial complex II (also known as succinate dehydrogenase, SDH) has a dual role in the electron transport chain and the TCA cycle, making a physical link between the two essential energy transduction processes of the cell. Dysfunction of this machinery underlies a number of human diseases, ranging from distinct tumour types to cardiomyopathy and neuromuscular disease and SDH subunits are categorised as tumour-suppressor proteins. In order to better understand the molecular details of the role of SDH as tumour suppressor, we knocked out/depleted subunits of CII using genomic editing and RNAi in breast cancer cell lines and studied their functional significance in relation to bioenergetics, supercomplex assembly, and tumour formation. Our results point to a new role of complex II, which is likely to have importance for the (patho)physiology of a cell.

CS12.03

CONCURRENT SESSION 12: RAPID FIRE PRESENTATIONS - CANCER ORIGINS AND TREATMENT
July 19, 2016 10:45 – 12:15

FHIT in Concert with p53 Modulates EGF-Dependent Cell Growth via Akt and COX-2 Signaling Crosstalk in MCF-7 Cells

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Abstract: Deregulation of oncogenic signaling pathways is an inevitable incident, conferring growth advantage to breast tumor cells. By the same token, aberration of tumor suppressor genes can amplify survival signaling transmission evoked by EGFR activation. Fragile Histidine Triad (FHIT) and p53 are key tumor suppressors that are affected upon EGFR activation. On the other hand, Akt and Cyclooxygenase-2 (COX-2) are crucial oncogenes which can be activated following growth factor stimulation. Considering EGFR as a common upstream molecule for these signaling modules, we pursued their functional crosstalk upon EGF treatment in MCF-7 cells. Cell viability was assessed using MTT. Protein expression was measured using Western Blotting in a time-course study. FHIT and p53 expression was silenced using RNAi. Results revealed that EGF biphasically altered the expression pattern of FHIT and p53, so that low-dose EGF increased either FHIT or p53 expression, whereas high-dose lowered the expression levels of the both. On the other hand, EGF dose- and time-dependently induced Akt phosphorylation; however, COX-2 expression level was unaltered in wild-type cells. Knock-down p53 expression completely reversed FHIT expression pattern, remodeled Akt activation profile, and induced COX-2 expression in a dose-dependent manner. Intriguingly, FHIT abrogation caused p53 down-regulation, enhanced early Akt signaling activation, and drastically induced COX-2 expression upon EGF treatment. Our findings give insights into a signaling convergence between FHIT and p53 pathways affecting EGFR-mediated cell growth via modulating Akt and COX-2 signaling activation, which can be targeted for manipulating cell proliferation and therapeutic approaches in breast cancer.

CS12.04

CONCURRENT SESSION 12: RAPID FIRE PRESENTATIONS - CANCER ORIGINS AND TREATMENT
July 19, 2016 10:45 – 12:15

UV Light-Inactivated HSV-1 Stimulates Natural Killer Cell-Induced Killing of Prostate Cancer Cells

Ismael Samudio[1], Elyse Hofs[2], Brandon Cho[2], Luke Bu[3], Guoyu Liu[3], Vivian Lam[4], Ingrid Elisia[4], Paul Rennie[5], William Jia[3], Gerald Krystal[4]
[1]Immunology, The Centre for Drug Research and Development, Vancouver, Canada; [2]Terry Fox Lab, British Columbia Cancer Agency, Vancouver, Canada; [3]Brain Research Centre, UBC, Vancouver, BC, Canada; [4]Terry Fox Lab, British Columbia Cancer Agency, Vancouver, BC, Canada; [5]Prostate Centre, Jack Bell Research Centre, Vancouver, Canada

Abstract: Herein we demonstrate that ultraviolet (UV) light-inactivated Herpes Simplex Virus-1 (UV-HSV-1) directly stimulates peripheral blood mononuclear cells (PBMCs) to destroy prostate cancer (PrCA) cell lines, but not allogeneic lymphocytes, and is 1000 to 10,000 fold more potent at stimulating this killing than UV-inactivated vesicular stomatitis virus, cytomegalovirus, adenovirus or reovirus. Amongst PBMCs, natural killer (NK) cells appear to be a major cell type involved in this killing and UV-HSV-1 appears to directly and potently stimulate expression of CD69, degranulation, migration, and cytokine production in these NK cells. These results suggest that components of the viral structure directly activate NK cells and this, together with our finding that UV-HSV-1 activated NK-induced killing of PC3 cells is markedly reduced by NFκB inhibition, supports the notion that Toll-like receptor (TLR) ligands on UV-HSV-1 are responsible via activation of NK cells via their TLRs. Curiously, we also found that UV-HSV-1 stimulates glycolysis in PBMCs, and that 2-deoxyglucose abrogates UV-HSV-1 activated killing by PBMCs, highlighting the importance of switching from oxidative phosphorylation to glycolysis in this anti-PrCA innate immune response. Importantly, UV-HSV-1 cooperates with IL-15 in inducing cytolytic activity of PBMCs against PrCA cell lines. Taken

together, our results support the preclinical development of UV-HSV-1 as an adjuvant, alone or in combination with IL-15, for NK cell infusions to treat PrCA.

CONCURRENT SESSION 13 | CS13
REGULATION OF STEM CELLS

CS13.01

CONCURRENT SESSION 13: REGULATION OF STEM CELLS
July 19, 2016 13:45 - 15:15

β-Catenin Is Required for T Cell Leukemia Initiation and MYC Transcription Downstream of Notch1

Anna Bigas
Program In Cancer Research, institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain

Abstract: Notch activation is instrumental in the development of most T-cell acute lymphoblastic leukemia (T-ALL) cases, yet Notch mutations alone are not sufficient to recapitulate the full human disease in animal models. We have found that Notch1 activation at the fetal liver stage expands the hematopoietic progenitor population and confers it transplantable leukemic initiating capacity. However, leukemogenesis and Leukemic Initiating Cell (LIC) capacity induced by Notch1 is critically dependent on the levels of β-Catenin, in both fetal liver and adult bone marrow contexts. By using different genetic mouse models and human cell lines, we have shown that β-Catenin essentially contributes to the leukemogenic potential of Notch in T-ALL by impinging on MYC gene activation. All three proteins, Notch, β-Catenin and MYC, have been previously found to play critical roles in the maintenance of leukemic stemness in T-ALL. We now demonstrate that Notch and β-Catenin are both recruited to the 3' MYC enhancer to control MYC transcription. In addition, we demonstrate that β-Catenin levels determine the number of leukemic initiating cells in a Notch1-dependent T cell leukemia mouse model, and abrogation of β-Catenin activity suppresses mouse and human T-ALL cell growth in vitro and in vivo.

CS13.02

CONCURRENT SESSION 13: REGULATION OF STEM CELLS
July 19, 2016 13:45 - 15:15

Cell Type-Specific Regulation by Intragenic CpG Islands

Young-Joon Kim
Yonsei Genome Institute, Seoul, Korea, Republic of

Abstract: CpG islands (CGIs) have long been known for their association with enhancers, silencers, and promoters, and for their epigenetic signatures. They are maintained in embryonic stem cells (ESCs) in a poised but inactive state, by the formation of bivalent chromatin, containing both active and repressive marks. CGIs also occur within coding sequences, where their functional role has remained obscure. Intragenic CGIs (iCGIs) are largely absent from housekeeping genes, but they are found in all genes associated with organ development and cell lineage control. We have investigated the epigenetic status of iCGIs and found that they too reside in bivalent chromatin in ESCs, with important differences from enhancers, silencers and promoters. iCGIs bind cell type-specific transcription factors, and they resolve upon cell differentiation by hypermethylation, spreading to promoters, and resulting in active transcription. iCGIs thus play a key role in the cell type-specific regulation of transcription.

CS13.03

CONCURRENT SESSION 13: REGULATION OF STEM CELLS
July 19, 2016 13:45 - 15:15

mTOR Independent Regulation of Mitochondrial Metabolism and Autophagy by Akt

Evan Dale Abel
Foe Diabetes Research Center, University of Iowa, Carver College of Medicine, Iowa City, United States of America

Abstract: A switch from glycolysis to oxidative phosphorylation is a characteristic facet of cellular differentiation. Activation of Akt has long been known to promote glycolysis by increasing cellular glucose uptake. We show that in cardiomyocytes Akt activation not only promotes glycolysis, but also represses mitochondrial oxidative phosphorylation via transcriptional mechanisms that are independent of mTOR. Akt activation also represses autophagy, via multiple mechanisms including mTOR-mediated suppression of autophagy and by phosphorylating Beclin1. We have identified a novel role for Akt in autophagy regulation whereby dephosphorylated Akt activates autophagy by forming a complex with Atg13 and HSP90b. This mechanism of autophagy regulation by Akt is independent of mTOR. These observations identify novel functionalities of Akt in the regulation of cellular metabolism and autophagy that may have implications for cellular differentiation.

CONCURRENT SESSION 14 | CS14
CELL DEATH AND CELL SURVIVAL

CS14.01

CONCURRENT SESSION 14: CELL DEATH AND CELL SURVIVAL
July 19, 2016 13:45 - 15:15

Galectin-3 Determines the Survival Strategy of Tumor Cells in Stressed Microenvironments

Roger Chammas
Center For Translational Cancer Research, Universidade de São Paulo, Sao Paulo, Brazil

Abstract: Galectin-3 is a member of the β-galactoside binding lectin family, whose expression is often dysregulated in cancers. While galectin-3 is usually an intracellular protein, found in the nucleus and in the cytoplasm; under certain conditions, galectin-3 can be secreted by an yet unknown mechanism. Under stressing conditions (hypoxia and nutrient deprivation, e.g.) galectin-3 is upregulated. Galectin-3 serves as a scaffold protein, which favors the spatial organization of signaling proteins as K-RAS, favoring cell survival. Upon secretion, extracellular galectin-3 interacts with a variety of cell surface glycoproteins, such as growth factor receptors, integrins and extracellular matrix molecules. Through its ability to oligomerize, galectin-3 forms lectin lattices that act as scaffolds that sustain the spatial organization of signaling receptors on the cell surface, dictating its maintenance on the plasma membrane or their endocytosis. Galectin-3 induces tumor cell, endothelial cell and leukocyte migration, favoring either the exit of tumor cells from a stressed microenvironment or the entry of endothelial cells and leukocytes, such as monocyte/macrophages into the tumor organoid. Therefore, galectin-3 plays homeostatic roles in tumors, besides its effects in different elements of the immune system, as (i) it favors tumor cell adaptation for survival in stressed conditions; (ii) upon secretion, galectin-3 induces tumor cell detachment and migration; (iii) it attracts endothelial cells and monocytes/macrophages to the tumor mass, inducing both directly and indirectly the process of angiogenesis. These activities are potentially targetable and specific interventions may be designed to counteract the protumoral role of galectin-3. Supported by FAPESP and CNPq.

CS14.02

CONCURRENT SESSION 14: CELL DEATH AND CELL SURVIVAL
July 19, 2016 13:45 - 15:15

Mechanisms Protecting Tumor Cells from DNA Damage Induced by Chemotherapeutic Agents

Carlos F. Menck, Alexandre Vessoni, Luciana R. Gomes, Clarissa R. Rocha
Of Microbiology, Institute of Biomedical Sciences University of Sao Paulo, Sao Paulo, Brazil

Abstract: Tumor therapy involves, in most cases, drugs that induce DNA damage, and different pathways process those lesions leading to tumor resistance. We investigated how glioma and breast cancer cells respond to genotoxic agents such as temozolomide (TMZ), chloroethylating agents, cisplatin (CP) and doxorubicin (DOXO). TMZ and CP damage DNA, although we observed that levels of glutathione on tumor cells play decisive roles on cell resistance. Modulating glutathione levels with BSO (a glutathione inhibitor) or NAC (a glutathione precursor) demonstrate cell protection from DNA damage and cell death. Also, using *in vivo* nude mice model, the combination of BSO and low doses of TMZ and CP were shown to inhibit tumor progression. Moreover, NFR2, a transcription factor that controls the cells responses to oxidative stress, is upstream controlling cells resistance to these agents. On the other hand, the influences of extracellular matrix (ECM) microenvironment and three-dimensional (3D) cell morphology were investigated on how human breast cancer cells respond to DOXO. Autophagy was compromised in the 3D cultures, and this results in the increased sensitivity to DOXO. Moreover, autophagy inhibition potentiated DOXO-induced cell death only in cells maintained under 2D culture conditions, while the autophagy inducer rapamycin improved the resistance of 3D-cultured cells to this drug. We further demonstrated that autophagy increase observed in monolayer cells depends on the p53- and DRAM-1 expression. Therefore, 3D tissue microenvironment controls breast-tumor cell sensitivity to DOXO treatment by preventing p53-DRAM-autophagy axis activation.
Financial Support: CNPq (Brasília, DF, Brazil) and FAPESP (São Paulo, SP, Brazil)

CS14.04

CONCURRENT SESSION 14: CELL DEATH AND CELL SURVIVAL
July 19, 2016 13:45 - 15:15

Proteasome Activation as a Novel Anti-Aging Strategy

Efstathios Gonos
National Hellenic Research Foundation, Athens, Greece

Abstract: Aging and longevity are two multifactorial biological phenomena whose knowledge at molecular level is still limited. We have studied proteasome function in replicative senescence and cell survival (Mol Aspects Med 35, 1-71; Ageing Res Rev 23, 37-55). We have observed reduced levels of proteasome content and activities in senescent cells due to the down-regulation of the catalytic subunits of the 20S complex (J Biol Chem 278, 28026-28037). In support, partial inhibition of proteasomes in young cells by specific inhibitors induces premature senescence which is p53 dependent (Aging Cell 7, 717-732). Stable over-expression of catalytic subunits or POMP resulted in enhanced proteasome assembly and activities and increased cell survival following treatments with various oxidants. Importantly, the developed "proteasome activated" human fibroblasts cell lines exhibit a delay of senescence by approximately 15% (J Biol Chem 280, 11840-11850; J Biol Chem 284, 30076-30086). Moreover, additional findings indicate that the recorded proteasome activation by many inducers is Nrf2-dependent (J Biol Chem 285, 8171-8184). Finally, we provide evidence that proteasome activation is an evolutionary conserved

mechanism, as it can delay aging in vivo and, importantly, it also confers deceleration of aggregation-related pathologies, such as Alzheimer's or Huntington's diseases (FASEB J 29, 611-622). Given these findings, recent work has identified a proteasome activator that decelerates aging and Alzheimer's disease (Antiox. Redox Signal, in press).

CONCURRENT SESSION 15 | CS15
REGULATION BY PHOTORESPONSES AND IONS

CS15.01

CONCURRENT SESSION 15: REGULATION BY PHOTORESPONSES AND IONS
July 19, 2016 13:45 – 15:15

Linking Light Perception and Stress Responses in the Filamentous Fungus Trichoderma Atroviride

Alfredo Herrera-Estrella[1], Víctor A. Correa-Pérez[2], Mónica García Esquivel[1], Elizabeth Medina-Castellanos[1], Edgardo U. Esquivel-Naranjo[2]
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Abstract: Most living organisms use sunlight as a source of energy and/or information about their environment. In the fungus *T. atroviride* blue-light is perceived through the Blue Light Regulator Complex, which regulates gene expression leading to the production of asexual reproduction structures. High-throughput RNA sequencing allowed us to identify 331 transcripts regulated by light and to demonstrate the existence of Blr1-dependent and independent responses. The set of light induced genes was enriched in stress related genes, which are also regulated by light in other fungi. Among this set we found genes encoding components of a stress activated MAPK signaling-pathway (SAPK). Mutants affected in two of the main components of this pathway (Δ*pbs2* and Δ*tmk3*) were highly sensitive to osmotic and oxidative stress, as well as UV irradiation. Interestingly, under oxidative stress the SAPK pathway showed specific roles during development, which in conidia are essential for tolerance to oxidant agents and appear to play a minor role in mycelia. The function of this pathway was more evident in Δ*pbs2* and Δ*tmk3* mutant strains when combining oxidative stress or cell wall damage with light. Strikingly, photoconidiation and expression of blue light regulated genes was severally affected in the Δ*tmk3* and Δ*pbs2* strains, indicating that this pathway regulates light responses. Furthermore, Tmk3 was rapidly phosphorylated upon light exposure. We propose a model in which Tmk3 cooperates with the Blr photoreceptor complex in the activation of gene expression that may involve its direct participation in the activation of gene expression.

CS15.02

CONCURRENT SESSION 15: REGULATION BY PHOTORESPONSES AND IONS
July 19, 2016 13:45 – 15:15

Signalling Networks of Plant Photo-Sensory Receptors

Jorge Casal
IFEVA, Buenos Aires, Argentina

Abstract: Phytochromes and Morphogenesis

CS15.03

CONCURRENT SESSION 15: REGULATION BY PHOTORESPONSES AND IONS
07-19-2016 13:45 – 15:15

Non-canonical Functions of the Beta-secretase (BACE1)

Filip Liebsch, Gerhard Multhaup
Pharmacology, McGill, Montreal, Canada

Abstract: BACE1 is an aspartic acid protease that belongs to a group of enzymes known as "sheddases". Responsible for releasing the ectodomain of several integral membrane proteins into the extracellular space, "ectodomain shedding" is regarded as an important regulatory process for the proper function of a variety of transmembrane proteins *in vivo*. The canonical enzymatic function of BACE1 has been implicated in diverse physiological processes. Overall, the regulation and "sheddase" function of BACE1 is linked to multiple substrates and a variety of important cellular processes in health and disease (e.g. amyloid deposition and neurodegeneration in rapidly aging "baby boomer" population). Importantly, new linkages between BACE1 and copper (Cu) metabolism have emerged. As an essential bioactive trace metal, altered Cu metabolism has been implicated in both physiological and pathophysiological aging. We recently discovered that Cu ions can bind to the transmembrane sequence (TMS) of BACE1, akin to a high affinity binding site for Cu(I) of Cu-transporting proteins. We now report that full-length BACE1 is predominantly trimeric in human cells and BACE1 in human cells has the ability to regulate cytosolic Cu concentrations via its TMS. Our results demonstrate that BACE1 has a role in Cu homeostasis, likely in the maintenance of cytosolic Cu levels and plays even a role in Cu detoxification under conditions of high cytosolic Cu levels. Adding to existing physiological models, our findings provide novel insights about the atypical TMS of BACE1 and its non-enzymatic activities.

CONCURRENT SESSION 16 | CS16
RAPID FIRE PRESENTATIONS - NEURAL SYSTEMS AND DISEASE

CS16.01

CONCURRENT SESSION 16: RAPID FIRE PRESENTATIONS - NEURAL SYSTEMS AND DISEASE
July 19, 2016 13:45 – 15:15

HSP60 Plays a Regulatory Role in IL-1β Induced Microglial Inflammation via TLR4-p38 MAPK Axis

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National Brain Research Center, Gurgaon, India

Abstract: The role of IL-1β has been extensively studied in neurodegenerative disorders; however, molecular mechanisms underlying inflammation induced by IL-1β are still poorly understood. The objective of our study is the comprehensive identification of molecular circuitry involved in IL-1β induced inflammation in microglia through protein profilin To achieve our aim, we performed the proteomic analysis of N9 microglial cells with and without IL-1b treatment at different time points. Expression of HSP60 in response to IL-1b administration was checked by quantitative real time PCR, immunoblotting and immunofluorescence. Interaction of HSP60 with TLR4 was determined by co-immunoprecipitation. Inhibition of TLR4 was done using TLR4 inhibitor to reveal its effect on IL-1b induced inflammation. Further, effect of HSP60 knockdown and overexpression were assessed on the inflammation in microglia. Twenty one proteins were found to be differentially expressed in response to IL-1b treatment in N9 microglial cells. *In silico* analysis of these proteins revealed unfolded protein response as one of the most significant molecular functions and HSP60 turned out to be a key hub molecule. IL-1β induced the expression as well as secretion of HSP60 in extracellular milieu during inflammation of N9 cells. Our knockdown and over expression studies demonstrated that HSP60 increases the phosphorylation of ERK, JNK and p38 MAPKs in N9 cells during inflammation. We can conclude that IL-1β induces expression of HSP60 in N9 microglial cells that further augments inflammation via TLR4-p38 MAPK axis.

CS16.02

CONCURRENT SESSION 16: RAPID FIRE PRESENTATIONS - NEURAL SYSTEMS AND DISEASE
July 19, 2016 13:45 – 15:15

Coupling of NR2A-NMDA Receptors to CaMKII Reactivates Dendritic and Spine Growth in Mature Hippocampal Neurons

Fernando Bustos, Nur Jury, Estibaliz Ampuero, Sebastian Abarzúa, Martin Montecino, Brigitte Van Zundert, Lorena Varela-Nallar
Universidad Andrés Bello, Santiago, Chile

Abstract: NMDA receptor (NMDAR) subunits NR2A and NR2B play critical roles in synaptic plasticity. While NR1NR2B receptors are predominantly expressed in immature neurons and promote plasticity, NR1NR2A receptors are mainly expressed in mature neurons and induce circuit stability. How the different subunits regulate these processes is unclear, but this is likely related to the presence of their distinct C-terminal sequences that couple different signaling proteins. Calcium-calmodulin-dependent protein kinase II (CaMKII) is an interesting candidate as this protein can be activated by calcium influx through NMDARs and trigger a biochemical signaling cascade that regulate plasticity. Using mutant constructs (i.e. NR2B-RS/QD) or peptides (i.e. tatCN21) that specifically interfere with NR2B-CaMKII binding, we showed that the recruitment and interaction of CaMKII with the NR2B C-terminal is required to induce dendritogenesis in immature hippocampal cultures. While NR2A does not effectively bind CaMKII, coupling of NR2A with CaMKII through expression of the construct NR2AΔIN reactivates dendritogenesis in mature hippocampal cultures. Moreover, HSV-mediated transduction of NR2AΔIN in mature hippocampal neurons *in vivo* resulted in dendritic outgrowth and an increased number of immature spines. Our data indicate that dominated NR2A expression and the limited interactions of this subunit with CaMKII contribute to the restricted plasticity in the adult CNS.

CS16.03

CONCURRENT SESSION 16: RAPID FIRE PRESENTATIONS - NEURAL SYSTEMS AND DISEASE
July 19, 2016 13:45 – 15:15

Metabolic Changes Associated with the Development of Alzheimer's Disease

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[1]Dalhousie University, Halifax, NS, Canada; [2]Faculty of Medicine, Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada

Abstract: Background: The human brain has a high energy demand that is required for the maintenance of normal brain function; learning and memory, synaptic transmission, production and recycling of neurotransmitters. Because of this demand, energy metabolism is highly regulated in the brain, relying on both astrocytes and neurons. Changes in brain metabolism occur throughout the normal aging process allowing for beneficial adaptations however, in neurodegenerative diseases including Alzheimer's disease (AD), metabolic changes may contribute to neuronal dysfunction. **Methods:** To better understand the role energy metabolism plays in the development of AD, we generated a novel model of AD using 5XFAD mice with altered brain energy metabolism. Brains and blood were harvested at two week intervals after birth, for up to 8 weeks, prepared for analysis for metabolites, methylation profiling of circulating DNA and signalling pathways associated with mitochondria function. **Results:** Temporal metabolic profile of brains harvested from 5XFAD mice was significantly different from the metabolic profile of brains harvested from control mice. Expression of metabolic enzymes was reflective of metabolic changes. Signalling pathways associated with mitochondria function were altered in brains of 5XFAD mice

compared with control mice. **Conclusions:** Temporal alterations in brain energy metabolism were found in 5XFAD mice compared to control mice. We conclude that early changes in brain energy contribute to the development and progression of Alzheimer's Disease.

CONCURRENT SESSION 17 | CS17
CANCER CELLS AND KINASES

CS17.01

CONCURRENT SESSION 17: CANCER CELLS AND KINASES
July 20, 2016 10:45 – 12:15

Integrin Linked Kinase (ILK) in Development and Disease

Shoukat Dedhar
Integrative Oncology, BC Cancer Research Centre, Vancouver, BC, Canada

Abstract: Integrin-Linked Kinase (ILK) is a ubiquitous focal adhesion protein that links the extracellular matrix (ECM), via integrins to the cytoskeleton and downstream signaling pathways. ILK is composed of ankyrin repeats, a plekstrin homology (PH) domain and an atypical kinase catalytic domain. Genetic knockout studies have demonstrated an essential role of ILK during blastocyst implantation, and conditional knock out of ILK in various tissues has confirmed essential roles in tissue homeostasis. ILK mutations in cardiomyocytes lead to dilated cardiomyopahy. ILK protein expression is elevated in most solid tumors as well as in acute and chronic myelogenous leukemia, where it plays a critical role in leukemic stem cells. ILK has been shown to actvate several oncogenic signaling pathways, including Pi3Kinase/Akt, Wnt, and NFkB. Overexpression of ILK also leads to epithelial to mesenchymal transition (EMT), and ILK is a critical component of TGF-b-driven EMT. Recent work from our laboratory has demonstrated that ILK silences the Hippo tumor supressor pathway by inactivating Merlin via phosphorylation-dependent inactivation of MYPT-1 (Serrano et al, Nature Comm, 2013) ILK has a complex interactome that localizes it, through interactions with PINCH, parvin and paxillin, to focal adhesions, and via distinct set of interacting proteins to centrosomes where it palys a distinct role in mitotic spindle assembly. ILK is activated by direct interaction with LIMD2, a LIM-domain only protein that binds to the catalytic domain and stimulates ILK kinase activity. This interaction and activation may be critical for tumor metastasis, since LIMD2 expression is enriched in metastases.

CS17.02

CONCURRENT SESSION 17: CANCER CELLS AND KINASES
July 20, 2016 10:45 – 12:15

Regulation of Tumorigenesis by Stress Kinase Signaling

Angela R. Nebreda
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Abstract: The protein kinase p38a is an important regulator of the stress response but can also integrate signals that affect many other cellular processes in a cell context- and cell type-specific manner. Studies using genetically modified mice have elucidated in vivo functions for p38a, and provided insights into how this pathway can suppress tumor initiation. There is evidence that normal cells rely on p38a signaling to engage various tumor suppressor mechanisms, including cell cycle arrest and apoptosis. However, p38a does not seem to be usually mutated in human tumors, and this signaling pathway sometimes seems to acquire new roles that facilitate tumor development. Using mouse models of cancer, we have obtained genetic evidence that transformed epithelial cells rely on the p38a pathway for survival and proliferation. Experiments using chemical inhibitors support a role for p38a signaling in tumor development *in vivo*. We are also investigating how the p38a pathway in stromal cells contributes to tumorigenesis. I will present results showing that myeloid cells rely on p38a signaling to support tumor progression by several mechanisms.

CS17.03

CONCURRENT SESSION 17: CANCER CELLS AND KINASES
July 20, 2016 10:45 – 12:15

Multidimensional Screening Identifies Positive and Negative Regulators of the Hippo Pathway

Liliana Attisano, Emad Heidary Arash, Ahmed Shiban, Siyuan Song, Ki M. Song, Mandeep Gill
Biochemistry, University of Toronto, Toronto, ON, Canada

Abstract: The Hippo signalling pathway is a key regulator of tissue growth and organogenesis. The pathway is comprised of a core MST/LATS kinase cassette which phosphorylates and promotes cytoplasmic localization of the transcriptional regulators, TAZ and YAP. To uncover novel Hippo pathway regulators, we conducted multidimensional high throughput screens involving LUMIER-based protein-protein interaction mapping, functional cDNA overexpression and siRNA knockdown assays using TAZ/YAP transcriptional reporters and imaging-based screens to examine subcellular localization of TAZ/YAP. These screens have uncovered several kinases that function to block Hippo pathway activity, such as the MARK kinase family, that we show functions to inhibit the Hippo pathway and promote TAZ/YAP activity in breast cancer cells. We also uncovered several positive Hippo/TAZ/YAP regulators such as ARHGEF7, commonly known as βetaPIX, a multidomain-containing guanine nucleotide exchange factor (GEF). We show that the βetaPIX binds both LATS and

TAZ/YAP to promote LATS-mediated repression of TAZ/YAP. BetaPIX functions downstream of both high cell density and actin cytoskeletal rearrangements and loss of βetaPIX expression in normal mammary epithelial cells strongly reduces TAZ/YAP phosphorylation, promotes nuclear localization and increases target gene expression. Increased expression of βetaPIX, in a triple negative breast cancer cell line which displays constitutively nuclear TAZ/YAP, recouples the Hippo kinase cassette to regulation of TAZ/YAP localization and inhibits cell migration and proliferation. Thus, our screens have identified both positive and negative regulators of the Hippo pathway that function in normal and tumorigenic breast cancer cell lines.

CS17.04

CONCURRENT SESSION 17: CANCER CELLS AND KINASES
July 20, 2016 10:45 – 12:15

Cysteine Phosphorylation and Magnesium Homeostasis

Kalle Gehring
Biochemistry, McGill University, Montreal, Canada

Abstract: The PRLs (Phosphatases of Regenerating Liver) are members of the PTP (protein tyrosine phosphatase) superfamily, and implicated in tumor growth and metastasis. The three PRL phosphatases (PRL1-3) are highly oncogenic and overexpressed in many cancers, yet their mechanism of action remains unknown. Recently published studies have suggested that PRL oncogenicity is mediated through the activity of a family of magnesium transporters, CNNM [1,2]. Structurally, PRLs bear similarity to the dual specificity phosphatases with a conserved, catalytic cysteine that acts as a nucleophile in catalysis. Our biophysical studies show that the interaction between PRLs and CNNMs is regulated by cysteine phosphorylation and oxidation. Crystallographic and functional studies reveal the structural basis of the interaction and potential upstream signals. These studies open the way for the design of therapeutic interventions to block the metastatic potential of PRL phosphatases.

CONCURRENT SESSION 18 | CS18
APOPTOSIS

CS18.01

CONCURRENT SESSION 18: APOPTOSIS
July 20, 2016 10:45 – 12:15

Cytochrome C: A Wolf in Sheep's Clothing

Irene Díaz-Moreno, Antonio Díaz-Quintana, Katiuska González-Arzola, Sofía M. García-Mauriño, Alejandra Guerra-Castellano, Carlos A. Elena-Real, Francisco Rivero-Rodríguez, Miguel Á. De La Rosa
Ciccartuja, University of Seville - CSIC, Sevilla, Spain

Abstract: Cytochrome *c* (Cc) is a soluble hemeprotein with a pleiotropic role in cell life and death. Under homeostasis, Cc is retained inside the mitochondria and performs gliding mechanisms to shuttle electrons between respiratory supercomplexes. Upon apoptotic stimuli, however, Cc is released into the cytoplasm, so letting Cc bind to Apaf-1 – the only reported extra-mitochondrial Cc target for many years - and assembling the apoptosome for activation of caspases. Interestingly enough is that apoptosis remains active in Apaf-1 knockout mutants but not in Cc knockout mutants, leading one to wonder if extra-mitochondrial Cc could play other possible functions. To go deeper in understanding the role of Cc in the onset of apoptosis and to harmonize the different phenotypes of Apaf-1 and Cc knockout mutants, we have proposed that the extra-mitochondrial Cc interacts with nuclear and/or cytoplasmic pro-survival, anti-apoptotic proteins, so as to lead living cells to dye. The histone chaperone SET/TAF-Iβ becomes a nuclear target of extra-mitochondrial Cc. In cell nuclei, Cc competitively hinders the binding of SET/TAF-Iβ to core histones, thereby locking its histone binding domains and inhibiting its nucleosome assembly activity. Furthermore, the post-transcriptional regulation of the mRNA encoding for Cc, along with the post-translational modifications of the hemeprotein, mainly by phosphorylation, open a new way to understand the Cc-dependent signaling upon homeostatic or apoptotic stimuli. *Acknowledgements:* This study was funded by the Spanish Ministry of Economy and Competiveness (Grant No. BFU2012-31670/BMC and BFU2015-71017/BMC), the Areces´ Foundation and the Regional Government of Andalusia (BIO198 and Grant No. P11-CVI-7216).

CS18.02

CONCURRENT SESSION 18: APOPTOSIS
July 20, 2016 10:45 – 12:15

Apoptosis

Xiao Dong Wang
National Institute of Biological Sciences, Beijing, China

Abstract: Xiao Dong Wang; Apoptosis; National Inst Bio Sciences, Beijing, China

CS18.03

CONCURRENT SESSION 18: APOPTOSIS
July 20, 2016 10:45 – 12:15

Anti-Apoptotic BAG-1 Plays a Role in the Phosphorylation of BAD to Mediate Cell Survival in Breast Cancer Cells

Gizem Dinler Doganay, Tugba Kizilboga, Salih Demir
Molecular Biology and Genetics Department, Istanbul Technical University, Istanbul, Turkey

Abstract: BAG-1 (Bcl-2 associated athanogene) is a multifunctional protein that interacts with diverse array of cellular targets and modulates a wide range of cellular processes, including

proliferation, cell survival, transcription, apoptosis, metastasis and motility. In human cells BAG-1 exists as three major isoforms (BAG-1S, BAG-1M, BAG-1L) derived by alternative translation initiation from a single mRNA, which allows interactions with various molecular targets such as Hsp70/Hsc70 molecular chaperones, components of the ubiquitylation/proteasome machinery, Bcl-2, Raf-1 kinase, nuclear hormone receptors and DNA. Overexpression of BAG-1 isoforms was shown to affect the regulation of proliferation, cell survival and cancer progression. There are number of possible mechanisms by which BAG-1 may mediate its pro-survival effects. In addition, drug resistance is observed with overexpression of BAG-1, causing cell rescue from apoptosis. Our work aims to investigate how altered BAG-1 expression levels affect cell survival pathways in various breast cancer and immortalized breast epithelial cells (MCF-7, MDA-MB231 and MCF-10A, respectively). To do that, we altered the expression levels of *BAG-1* gene in cells and checked relative expression levels of BAG-1, its interacting partners and certain proteins which are important for survival pathway by western blot analysis and immunocytochemistry. We performed immunoprecipitation to show co-existence of certain proteins with BAG-1 protein as a complex. Our results suggest that once BAG-1 forms a complex with C-Raf/B-Raf/Hsp70/Akt/Bcl-2, phosphorylation of Bad from Ser136 occurs and this leads to Bad's sequestration by 14-3-3, modulating cell survival. This work is supported by Istanbul Technical University Internal Funds

CONCURRENT SESSION 19 | CS19
METABOLIC SIGNALING

CS19.01

CONCURRENT SESSION 19: METABOLIC SIGNALING
July 20, 2016 10:45 – 12:15

The GRK2 Signaling Hub in the Molecular Physiopathology of Obesity-Related Diseases

Federico Mayor, Rocio Vila-Bedmar, Marta Cruces-Sande, Elisa Lucas, Alba C. Arcones, Cristina Murga
Centro De Biología Molecular "severo Ochoa", Universidad Autónoma Madrid, Madrid, Spain

Abstract: G protein-coupled receptor kinase 2 (GRK2), a known regulator of GPCRs, is emerging as a key player in insulin resistance (IR)-related conditions such as diabetes, obesity, non-alcoholic fatty liver disease (NAFLD) and cardiovascular pathologies. GRK2 levels are elevated in adipose tissue, muscle and liver in murine models and in lymphocytes from IR patients, negatively modulating insulin signalling by interacting with IRS1. Conversely, an IR and obesity phenotype is prevented in GRK2+/- hemizygous mice (Garcia-Guerra et al, Diabetes, 2010), which display enhanced energy expenditure (Vila-Bedmar et al, FASEB J, 2012). Inducible GRK2 deletion during a high-fat-diet (HFD) reverts key aspects of the obese phenotype in mice (body weight, glucose intolerance, insulin sensitivity, fat mass, liver steatosis, inflammation) (Vila-Bedmar et al, Science Signaling, 2015). Ongoing research describes a role for GRK2 in the development of NAFLD and that GRK2 dosage in macrophages is relevant for triggering the IR phenotype. Notably, cardiac GRK2 also increases in IR models, whereas GRK2+/- mice display enhanced cardiac insulin sensitivity and a cardioprotective gene expression reprogramming (Lucas et al, BBA Mol Bas Dis, 2014). Sympathetic over-activation and HFD appear to converge in upregulating GRK2, leading to altered adrenergic and insulin signalling, dysregulated metabolism and maladaptive myocardial remodelling. We postulate that GRK2 acts as a key signalling hub for IR in different cell types due to its unique ability to modulate the insulin cascade, key GPCRs controlling energy homeostasis and other signaling proteins related to IR conditions, thus emerging as a target for the treatment of IR-related diseases.

CS19.02

CONCURRENT SESSION 19: METABOLIC SIGNALING
July 20, 2016 10:45 – 12:15

Lipids and mTOR Signaling

Patricia Bozza
FIOCRUZ, Brazil, Brazil

CS19.04

CONCURRENT SESSION 19: METABOLIC SIGNALING
July 20, 2016 10:45 – 12:15

Metformin, an Antidiabetic and Putative Anti-Cancer Drug, Inhibits Endoplasmic Reticulum Stress and Autophagy

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Pharmacology, Weill Cornell Medicine in Qatar, Doha, Qatar

Abstract: Metformin, in clinical use for the treatment of type 2 diabetes (T2D) for 60 years, is being investigated as an anti-cancer drug and in vitro studies suggest an anti-proliferative action. Arguably the anti-hyperglycaemic action of metformin is via AMPK-dependent signalling; however, the contribution of AMPK to the anti-cancer effects of metformin is controversial. Endoplasmic reticulum stress (ERS) and autophagy are pro-survival processes in cancer. We investigated the concentration-dependent effects of metformin on glucose starvation (GS)-induced ERS and autophagy in mouse microvascular endothelial cells (MMECs) overexpressing vascular endothelial growth factor (VEGF). 24h of GS (compared to normal glucose) increased levels of the protein markers of ERS: GRP78 = x18; ATF4 = x9 and CHOP = x18. GS also increased the levels of markers of autophagy, LC3A-II (x3) and LC3B-II (x5), but the presence of 2mM, but not 50×M (peak therapeutic level of metformin for T2D), reversed the effects of GS. The mTOR pathway, which is negatively regulated by AMPK and plays an important role in the regulation of autophagy, was also inhibited by 2mM metformin. The role of AMPK was investigated using a siRNA knockdown and the AMPK activator, A769662. A769662, like metformin, inhibited the mTOR pathway and inhibited autophagy; however, knockdown of AMPK did not reduce the effects of

metformin. Furthermore, cell viability was reduced by metformin and induced cell-cycle arrest in G2/M. In conclusion, metformin via an AMPK-independent pathway, inhibits ERS and autophagy in GS-stressed cells thus providing a potential mechanism for the putative anti-cancer effects of metformin.

CONCURRENT SESSION 20 | CS20
RAPID FIRE PRESENTATIONS - CARDIAC AND INFLAMMATORY DISEASE

CS20.01

CONCURRENT SESSION 20 – RAPID FIRE PRESENTATIONS - CARDIAC AND INFLAMMATORY DISEASE
July 20, 2016 10:45 – 12:15

Early Elevation of H2S in Serum of ST Segment Elevation Myocardial Infarction (STEMI) Patients

Sara E. Ali[1], Mohamed A. Farag[2], Rasha S. Hanafi[3], Mohamed Z. Gad[4]
[1]Pharmaceutical Biology, The German University in Cairo, Cairo, Egypt; [2]Pharmacognosy, Faculty of Pharmacy - Cairo University, Cairo, Egypt; [3]Pharmaceutical Chemistry, The German University in Cairo, Cairo, Egypt; [4]Biochemistry, The German University in Cairo, Cairo, Egypt

Abstract: Background:Discovery of new biomarkers is critical for early diagnosis of coronary artery disease (CAD). Recent advances in metabolomic technologies have drastically enhanced our understanding of the mechanisms involved in the progression of this disease. **Methods and Results:** Serum metabolic profiling of ST-elevation myocardial infarction (STEMI), unstable angina (UA) and healthy controls was performed using GC/MS. A metabolite biosignature was developed that could robustly discriminate STEMI patients from controls and UA patients. Results showed that potential panel of biomarkers consisting of fourteen metabolites were identified in serum of STEMI patients. One of the most intriguing findings among these biomarker metabolites that merit further investigation is hydrogen sulfide (H_2S), an endogenous signaling gasotransmitter with profound effect on the heart and circulation. Serum H_2S was further assessed using a quantitative ELISA technique. This highly sensitive immunoassay confirmed the elevation of H_2S in serum of STEMI patients, as early as two hours after incidence of chest pain. H_2S levels were also found elevated in UA patients. However, the increase was less pronounced than STEMI patients. **Conclusion:** Metabolomics could be useful as a screening tool for early diagnosis and prognosis, and provide an opportunity to develop predictive biomarkers that will potentially allow for earlier intervention. The early elevation of H_2S levels was able to discriminate between CAD patients and healthy controls. The considerable interest in the biology of H_2S has resulted in heightened enthusiasm for the clinical translation of this ephemeral gaseous molecule.

CS20.02

CONCURRENT SESSION 20 – RAPID FIRE PRESENTATIONS - CARDIAC AND INFLAMMATORY DISEASE
July 20, 2016 10:45 – 12:15

Characterizing Cardiac Actin Variants from Molecules to Organisms

Haidun Liu, Matiyo Ojehomon, Mary Henein, John F. Dawson
Molecular and Cellular Biology, University of Guelph, Guelph, Canada

Abstract: Mutations in the cardiac actin gene (ACTC) are related to the development of both hypertrophic and dilated cardiomyopathy (HCM and DCM). We have examined the impact of changes in the ACTC protein from molecules to whole animals. With recombinant human cardiac actin variant proteins purified using a baculovirus expression system, we show that some subdomain 1 variants impact the actomyosin contractile system and others do not. E99K and R95C ACTC increase the duty ratio of the actomyosin contractile system, suggesting that changes in overall force a primary cause of the resulting heart disease. In contrast, the H88Y and F90Δ ACTC variants do not impact actomyosin interactions. To connect molecular changes to alterations in physiology, we employed CRISPRs and transposons to knock out the cardiac actin genes in the zebrafish (Danio rerio) animal model and express human cardiac actin in zebrafish hearts, respectively. Our data demonstrate that different zebrafish cardiac genes are active at different developmental stages. The changes in morphology of the heart, circulation, and physiology of zebrafish as a result of cardiac actin knockouts and human cardiac actin expression will be presented. Taken together, our systems permit characterization of the impact of changes in cardiac sarcomere proteins at the molecular and physiological levels.

CS20.03

CONCURRENT SESSION 20 – RAPID FIRE PRESENTATIONS - CARDIAC AND INFLAMMATORY DISEASE
July 20, 2016 10:45 – 12:15

SIRT6 Regulates Hexokinase 2 Expression and its Function

Ellora Sen, Piyushi Gupta, Touseef Sheikh, Pruthvi Gowda
National Brain Research Centre, Manesar, India

Abstract: Tumor cells are dependent on the Warburg effect for their survival, and Hexokinase (HK2) -a key glycolytic enzyme is indispensable for exhibiting the Warburg effect. Dysregulated metabolism in Glioblastoma multiforme (GBM) - the most malignant of brain tumors, is characterised by enhanced HK2 levels. In addition to aberrant metabolism, inflammation is also an indispensable participant in GBM progression. We therefore investigated the role of pro-inflammatory cytokine IL-1b in regulating HK2 expression in glioma cells. IL-1β-induced increase in HK2 expression was concomitant with elevation in Sirtuin 6 (SIRT6) and Myeloid zinc finger-1 (MZF1) levels – both of which negatively regulated HK2 expression. While IL-1b decreased nucleosomal occupancy on HK2 promoter, ectopic SIRT6

expression induced chromatin compactness. IL-1β mediated nucleosomal re-organization was greatest in the region spanning -456bp to -693bp of the HK2 promoter containing putative MZF1 binding sites. SIRT6 over-expression induced interaction between MZF1/SIRT6 and facilitated recruitment of this complex to MZF1 site, thereby reconfiguring the chromatin structure that favours a regulatory state conducive to diminished transcription. Interestingly, IL-1b treatment under glucose deprivation induced (i) nuclear HK2 and SIRT6 accumulation, (ii) increased expression and activity of xanthine oxidoreductase (XOR), and (iii) apoptosis in ROS dependent manner. Though HK2 has no role in apoptosis, it regulated XOR expression. Our data highlight a mechanism whereby SIRT6 regulates HK2 expression and its ability to regulate gene involved in redox homeostasis.

CONCURRENT SESSION 21 | CS21
PARASITIC AND BACTERIAL DISEASE

CS21.01

CONCURRENT SESSION 21: PARASITIC AND BACTERIAL DISEASE
July 21, 2016 10:30 – 12:00

Endogenous RNA Viruses as Virulence Factors in Parasitic Protozoa

Stephen M. Beverley
Molecular Microbiology, Washington University School of Medicine, St. Louis, St. Louis, United States of America

Abstract: Virus-like elements occur in many parasitic protozoans but their significance was often a matter of conjecture. *Leishmania* species in South America often bear a dsRNA virus named LRV1. Like most Totiviruses, LRV1 is neither shed nor infectious, and may be seen as a persistent endobiont. We showed that in animal models, *Leishmania guyanensis* LRV1 is associated with hypervirulence and increased metastasis, the latter being a hallmark of more severe forms of leishmaniasis (Ives *et al., Science* 2011). We have been pursuing this observation as a new paradigm of protozoal virulence. We developed tools for reproducibly generating isogenic lines lacking LRV1s, allowing us to extend our findings to *L. braziliensisis*, the predominant agent of mucocutaneous leishmaniasis (MCL), one of the most severe forms. Transcriptomic analysis of infected macrophages shows an elevated 'hyperinflammatory' response including stimulation of many type I interferon-inducible genes. How these act to promote pathogenesis is now under study. Another question is the contribution of LRV1 to *Leishmania* pathogenicity in human infections, where disease manifestations differ greatly from those seen in murine models. Recently we showed the presence of LRV1 was associated with increased relapse and/or treatment failures in human *L. braziliensis*-infected patients treated with pentavalent antimonials in Peru and Bolivia (Aduai *et al., J. Infectious Diseases* 2016). The association of LRV1 with clinical drug treatment failure could serve to guide more effective treatment of leishmaniasis through anti-LRV1 directed therapies. I thank the members of my laboratory and that of Nicolas Fasel. Supported by grants from the US NIH.

CS21.02

CONCURRENT SESSION 21: PARASITIC AND BACTERIAL DISEASE
July 21, 2016 10:30 – 12:00

Non Canonical Inflammasome Activation During Mala

Ricardo T. Gazzinelli
Laboratory of Immunopathology, Fiocruz-Minas, Belo Horizonte, Brazil

Abstract: The inflammasomes are formed by a protein complex composed of cytoplasmic receptors such as NOD-Like Receptors (NLR), which is important for caspase-1 activation and consequent cleavage of pro-IL-1β into its active form. Previous works have shown that during malaria, induction and production of IL-1β depends on caspase-1 activation by the canonical pathway. Recently, several reports have demonstrated a non-canonicalactivation of caspase-1 mediated by capase-11. In the present work, we investigated the induction of the inflammatory caspases, caspase-11 and caspase-4/5 rodent and human respectively. We demonstrated that during malaria, caspase-11 and caspase-4/5 are induced and cleaved on their active form. We found that caspase-11 activation is dependent on INF-ϒ and endosomal TLR. However, caspase-11 is not necessary for caspase-1 activation. Moreover, caspase-$11^{-/-}$ mice were less susceptible to low dose LPS shock than infected WT mice. This was consistent with the lower IL-1β levels in the challenged caspase-$11^{-/-}$. We also found no evidence that caspase-11 is involved on induction of pro-IL-1β expression and that this induction requires TLR4 signaling. Hence, our results suggest that caspase-11 maybe favoring IL-1β secretion. In conclusion, we demonstrate that caspase-11 is induced and play an important role in the hypersensitivity to endotoxic shock during malaria.

CS21.03

CONCURRENT SESSION 21: PARASITIC AND BACTERIAL DISEASE
July 21, 2016 10:30 – 12:00

Signaling in Regulatory B Cell Induction in Trypanosoma Cruzi Infection

Adriana Gruppi
Bioquimica Clinica - Fcq-unc, CIBICI-CONICET, Cordoba, Argentina

Abstract: B lymphocytes (B cells) differentiate in plasma cells (PC) to provide essential protection against infections through antibody (Ab) production. In the last years, novel functions independent of Ab production have been described for B cells. Indeed, B cells act as drivers and/or regulators of immunity by presenting antigens and secreting cytokines. Immunoregulatory properties of B cells have been traditionally associated to IL-10 production. However, we reported that B cells and PC are a key source of IL-17 during *Trypanosoma cruzi* infection and that B lineage cell-derived IL-17 regulates inflammatory response during this parasite infection. We also identified that PC can exert regulatory function through inhibitory surface receptors. To dissect signaling pathways and transcriptional programs involved in the generation of regulatory PC might be of great significance for the development of new strategies aiming at the therapeutic targeting of B cells in the clinic.

CS21.04

CONCURRENT SESSION 21: PARASITIC AND BACTERIAL DISEASE
July 21, 2016 10:30 – 12:0**0**

Mechanistic Diversity in Antibiotic Resistance

Gerry Wright
McMaster University, Hamilton, ON, Canada

Abstract: Antibiotic Resistance

CONCURRENT SESSION 22 | CS22
METABOLIC SIGNALING AND DIABETES

CS22.01

CONCURRENT SESSION 22: METABOLIC SIGNALING AND DIABETES
July 21, 2016 10:30 – 12:0**0**

Role of Mitochondrial Dynamics on Metabolic Homeostasis

Antonio Zorzano
Molecular Medicine Program, IRB Barcelona, Barcelona, Spain

Abstract: Mitochondria and Diabetes

CS22.02

CONCURRENT SESSION 22: METABOLIC SIGNALING AND DIABETES
July 21, 2016 10:30 – 12:00

Caloric Restriction Increases Brain Mitochondrial Calcium Retention Capacity and Protects Against Excitotoxicity

Alicia Kowaltowski
Universidade de São Paulo, São Paulo, Brazil

Abstract: Caloric restriction (CR) protects against many cerebral pathological conditions that are associated with excitotoxic damage and calcium overload, although the mechanisms are still poorly understood. Here we show that CR strongly protects against excitotoxic insults in vitro and in vivo in a manner associated with significant changes in mitochondrial function. CR increases electron transport chain activity, enhances antioxidant defenses and favors mitochondrial calcium retention capacity in the brain. These changes are accompanied by a decrease in cyclophilin D activity and acetylation and an increase in Sirt3 expression. This suggests that Sirt3-mediated deacetylation and inhibition of cyclophilin D in CR promotes the inhibition of mitochondrial permeability transition, resulting in enhanced mitochondrial calcium retention. Altogether, our results indicate that enhanced mitochondrial calcium retention capacity underlies the beneficial effects of CR against excitotoxic conditions. This protection may explain the many beneficial effects of CR in the aging brain.

CS22.03

CONCURRENT SESSION 22: METABOLIC SIGNALING AND DIABETES
July 21, 2016 10:30 – 12:00

Control of Male-Female Differences in Growth by Sex Determination Gene Transformer during Development in Drosophila

Jason W. Millington, Elizabeth Rideout
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Abstract: Sex differences in body size exist in most animals, including humans. While sex hormones contribute to sex differences in body size, studies have shown that biological sex affects body size independently of sex hormones. However, the mechanisms underlying sex hormone-independent effects of sex on body size remain unclear. To tackle this question, we use *Drosophila melanogaster* larvae as a model. Larvae are ideal for these studies, possessing no sex hormones, identical food intake between the sexes, and conserved pathways that regulate growth. Our analysis of growth in males and females revealed a novel role for sex determination gene *transformer* (*tra*) in the regulation of body size. Tra protein is only expressed in females, which are larger, and not in males. We found that Tra expression in the fat body promotes insulin secretion from the insulin-producing cells in the brain. Higher levels of circulating insulin-like peptides in females promotes increased body size. To understand how Tra function in the fat body promotes insulin secretion and growth, we used RNA-seq to compare gene expression in larvae with and without Tra expression. We identified a total of 141 genes with differential expression, 14 of which are expressed in the fat body. Gene ontology analysis revealed 36% of genes with differential expression fell into 7 classes of metabolic processes. These results suggest a model of sex differences in body size in which Tra promotes specific aspects of cellular metabolism in the fat body to enhance insulin secretion, and consequently a larger female body size.

CS22.04

CONCURRENT SESSION 22: METABOLIC SIGNALING AND DIABETES
July 21, 2016 10:30 – 12:00

Anti-Steatotic Effect of Eicosapentaenoic Acid/Hydroxytyrosol Mixture in Liver of Mice Subjected to High Fat Diet

Rodrigo Valenzuela[1], Francisca Echeverria[1], Macarena Ortiz[2], Miguel Rincón[3], Maria Catalina Hernandez-Rodas[1], Cynthia Barrera[1], Macarena Marambio[1], Alfonso Valenzuela[1], Alejandra Espinosa[1], Luis Videla[1]
[1]Nutrition, University of Chile, SANTIAGO, Chile; [2]University of Chile, SANTIAGO, Chile; [3]Nutrition, University of Chile, SANTIAGO, Bermuda

Abstract: Introduction: Non-alcoholic fatty liver disease (NAFLD) is characterized by accumulation of lipids, insulin resistance,

oxidative stress, inflammation and depletion of n-3 LCPUFA. Eicosapentaenoic acid acid (EPA) control liver metabolism by stimulation of lipolysis and inhibition of lipogenesis. Hydroxytyrosol (HT) exhibits strong cytoprotective effects. **Aim:** To evaluate molecular mechanisms involved in protective effects of EPA / HT mixture, to prevent NAFLD induced by high fat diet (HFD) in mice. **Material and methods:** Male mice C57BL/6J received control diet (CD) (10% fat, 20% protein, 70% carbohydrate) or HFD, without and with supplementation with EPA (50 mg per kg per day) / HT (5 mg per kg per day) mixture for 12 weeks. **Results:** HFD produced (i) liver steatosis, (ii) higher serum glucose, insulin, HOMA index, total cholesterol, triacylglycerols, TNF-α, IL-6 and IL-1β, (iii) liver and plasma oxidative stress, (iv) depletion of liver n-3 LCPUFA, increment of lipogenic and reduction of lipolytic enzyme activites, (v) downregulation of PPAR-α and Nrf2, and upregulation of SREBP-1c and NF-kB. These changes were either reduced ($p < 0.05$) or normalized (compared to controls) in animals feed HFD and EPA / HT mixture. **Conclusion:** EPA / HT exerts anti-steatotic effects underling antioxidants and anti-inflammatory responses, improving insulin sensitivity and recovering liver lipolytic/lipogenic status altered by HFD, being PPAR-α and Nrf2 upregulation and SREBP-1c and NF-κB downregulation important mechanisms involved in this effects. Results support the potential therapeutic use of EPA / HT supplementation to treat NAFLD of dietary or other origin.
Acknowledgment: Supported by Grant 11140174 from FONDECYT.

CONCURRENT SESSION 23 | CS23
MEMBRANE TRANSPORT

CS23.01

CONCURRENT SESSION 23: MEMBRANE TRANSPORT
July 21, 2016 10:30 – 12:00

Harnessing the Biology of Glycosphingolipid Trafficking for Biologic Drug Delivery

Wayne Lencer, Dan Chinnapen
Pediatrics, Boston Childrens Hospital, Harvard Medical School, Boston, United States of America

Abstract: Mucosal surfaces and the vasculature represent vast areas where host tissues are separated from the environment or blood only by a delicate but highly effective single layer of epithelial or endothelial cells joined by tight junctions impermeable to proteins and even small peptides. So far, the lack of rational and efficient methods to circumvent these barriers has prevented the application of most therapeutic peptides and proteins for mucosal drug delivery. We have addressed these problems using certain GM1 glycosphingolipids as molecular carriers to deliver biologically active peptides across epithelial barriers by transcytosis. The founding principles are based on our earlier studies that show the structure of the ceramide domain of GM1 dictates intracellular trafficking of the sphingolipid. GM1 containing ceramide domains with short or unsaturated fatty acyl chains sort into recycling endosomes and across polarized epithelial cells by transcytosis. GM1 with long saturated fatty acyl chains sort into the lysosome. The GM1 lipids are fused via their extracellular oligosaccharide domain to reporter peptides (or to the incretin hormone GLP-1) yielding robust signals for tracking the molecules by imaging and biochemically. When applied to apical surfaces of epithelial cells in monolayer culture, only the peptides coupled to GM1-ceramides with short- or cis-unsaturated fatty acids traffic efficiently across the cell to the basolateral membrane by transcytosis. We modified the structure of the GM1 ceramide so that the efficiency of transport across epithelial barriers and release is now 10-70 fold higher than background. *In vivo* studies show absorption across the intestinal epithelial barrier.

CS23.02

CONCURRENT SESSION 23: MEMBRANE TRANSPORT
July 21, 2016 10:30 – 12:00

Arsenic Transport by Human MRP1 (ABCC1) is Selectively Modified by Phosphorylation and N-Glycosylation

Caley B. Shukalek[1], Diane P. Swanlund[1], Rodney K. Rousseau[1], Kevin E. Weigl[2], Vanessa Marensi[1], Susan P. Cole[2], Elaine M. Leslie[1]
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[2]*Department of Pathology and Molecular Medicine and Division of Cancer Biology and Genetics, Queen's University, Kingston, ON, Canada*

Abstract: Millions of people world-wide are chronically exposed to the environmental toxicant arsenic in drinking water. This has caused a public health crisis because arsenic is a proven human carcinogen. Arsenic is methylated in human cells and also forms glutathione (GSH/GS) conjugates. The ATP-binding cassette (ABC) transporter MRP1 (*ABCC1*) is responsible for the cellular export of a chemically diverse array of endogenous and xenobiotic metabolites. Using a membrane vesicular transport assay, we previously showed arsenic triglutathione [$As(GS)_3$] is transported by MRP1 expressed in HeLa cells with high affinity (*Km* 0.32 ×M) and low capacity (*Vmax* 42 pmol/mg protein/min). In contrast, we have now found that MRP1 expressed in HEK293 cells has a 12-fold higher *Km* and 7-fold higher *Vmax* for $As(GS)_3$ than that of MRP1 expressed in HeLa cells. Further investigation revealed that the substantial differences in $As(GS)_3$ transport kinetics are associated with differences in MRP1 post-translational modifications. Specifically, MRP1 affinity and capacity for $As(GS)_3$ vary depending on the glycosylation status of Asn19 and Asn23 and phosphorylation status of Tyr920 and Ser921. Our data indicate potential cross-talk between MRP1 *N*-glycosylation and phosphorylation, with phosphorylation dictating the affinity and capacity for $As(GS)_3$, and *N*-glycosylation influencing the phosphorylation status. Transport of other organic anions [e.g., 17β-estradiol 17-(β-D-glucuronide) and methotrexate] by MRP1 was unaffected by Tyr920/Ser921 phosphorylation, suggesting that this modulation is substrate-selective. These findings are significant because they suggest that Tyr920 and Ser921 phosphorylation can switch MRP1 to a lower affinity, higher capacity $As(GS)_3$ transporter, allowing arsenic detoxification over a broad concentration range.

CS23.03

CONCURRENT SESSION 23: MEMBRANE TRANSPORT
July 21, 2016 10:30 – 12:00

Oxysterol Binding Protein-Related Protein 1 (ORP1) is Required for Cholesterol Export from the Late Endosomes/Lysosomes

Neale Ridgway[1], Kexin Zhao[2]
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Abstract: Cells acquire cholesterol via de novo synthesis or receptor–mediated uptake of low-density lipoprotein (LDL). Cholesterol esters in LDL are degraded in the late endosomes/lysosomes (LELs) where the Niemann-Pick C1 (NPC1) and NPC2 proteins deliver the cholesterol to the limiting membrane of LELs. Cholesterol is then exported from LELs to other organelles, primarily the endoplasmic reticulum (ER) and plasma membrane. However, the identity of the protein(s) that exports cholesterol at this final stage is unknown. Here we report that oxysterol-binding protein-related protein 1 (ORP1), which includes ORP1L and ORP1S variants, catalyzes this final step in LDL-cholesterol transport. CRISPR-knockout of ORP1L in cultured cells inhibited cholesterol esterification by >60% and increased de novo cholesterol synthesis, indicative of reduced cholesterol export to the ER. The defect in cholesterol export to the ER was restored to wild-type levels by re-expression of ORP1L. Wild-type cells had dispersed LELs compared to the cholesterol-loaded LELs that clustered around the nucleus of ORP1L-null cells. CRISPR-knockout of both ORP1L and ORP1S did not further inhibit cholesterol export to the ER but caused a severe reduction in plasma membrane cholesterol content as measured with cholesterol-specific fluorescent probes. ORP1L/ORP1S-null cells also displayed enhanced cholesterol accumulation in LELs, and reduced LDL uptake and LDL receptor expression. The phenotype of ORP1L/ORP1S-null cells is similar to that observed in NPC1/2 deficiency, indicating a blockage in LDL-cholesterol export from the lysosomal/endosomal compartment. We conclude that ORP1L mediates cholesterol transport to the ER, while ORP1S transports cholesterol to the plasma membrane.

CS23.04

CONCURRENT SESSION 23: MEMBRANE TRANSPORT
July 21, 2016 10:30 – 12:00

Signaling Circuits Regulate PC-1 Exit from the Endoplasmic Reticulum Implications for the Therapy of Collagenopathies

Anita Capalbo[1], Rosaria Dimartino[1], Antonella Dicampli[1], Jorge Cancino[2], Alessandra Varavallo[1], Oreste Acuto[3], Benjamin Thomas[3], Alberto Luini[1]
[1]Institute of Protein Biochemistry, CNR, Napoli, Italy; [2]Universidad Andrés Bello, Vina del Mar, Chile; [3]Sir Dunn Pathology School, Oxford, United Kingdom

Abstract: Constitutive secretion is a fundamental cellular process by which 30% of human proteome is processed and transported from the site of synthesis, endoplasmic reticulum (ER) to their final appropriate cellular destination. The prevailing view for a long time in the field of membrane transport is that constitutive secretion is not regulated at all. Recently it has become clear that constitutive secretion is potently regulated by different kinases and signaling pathways at multiple stages of the pathway, with important physiological consequences. We now report that properly folded proteins are sensed in ER initiating a signaling reaction that leads to activation of the ER export machinery. Thus, the synchronous folding of Pro-Collagen type I trigger multiple signaling cascades, one of which results in activation of PKA, which controls cargo secretion. Through multiple approaches, we have identified cAMP/PKA ER compartmentalized machinery that comprises of specific isoforms of Adenylate Cyclase (AC), Phosphodiesterase (PDE) and A-Kinase-Anchoring-Protein (AKAP) at the ER membrane. We have demonstrated that ER cargo secretion can be regulated through the modulation of cAMP levels as well as PKA activation. We were also able to rescue the pathological PC-I ER retention in different human Collagenopathies derived cell lines. Our findings increase the knowledge on how cargo exits ER and can be applied to the development of therapeutic strategies to prevent the accumulation of proteins in ER, a common phenotype in many genetic human diseases.

CONCURRENT SESSION 24 | CS24
RAPID FIRE PRESENTATIONS - INFLAMMATORY DISEASE AND ECM

CS24.01

CONCURRENT SESSION 24 – RAPID FIRE PRESENTATIONS - INFLAMMATORY DISEASE AND ECM
July 21, 2016 10:30 – 12:00

Control of B Lymphocyte Activation and Migration by PI 3-Kinase: Role of Inositol Polyphosphate 4-Phosphatases

Aaron J. Marshall, Sen Hou, Xun Wu, Hongzhao Li
Immunology, University of Manitoba, Winnipeg, MB, Canada

Abstract: Balanced activation of the phosphoinositide 3-kinase (PI3K) pathway is required for effective immunity while avoiding pathological inflammation and lymphoproliferative diseases. PI3K generates the phosphoinositide PI(3,4,5)P3 which can then be converted to PI(3,4)P2 by the critical regulatory phosphatase SHIP. A number of key signaling molecules bind to PIP3, including the protein kinase Btk; however much less is known about the function of PI(3,4)P2. The inositol phosphatase INPP4A can specifically hydrolyze PI(3,4)P2; however the functions of this phosphatase in B cell activation are unknown. We hypothesized that INPP4A can provide a tool to deplete PI(3,4)P2 and determine its range of functions. Human B lymphocytes over-expressing active or phosphatase-dead INPP4A were generated, and the cells expressing active enzyme were found to generate lower levels of PI(3,4)P2 upon chemokine or BCR stimulation. Chemokine-induced migration responses were inhibited by active, but not inactive INPP4A. An assessment of BCR signaling was carried out that compared BCR-stimulated cells expressing active versus inactive INPP4A in a screen encompassing over 800 protein

phosphorylation sites. The results indicate that active INPP4A can suppress phosphorylation of Akt and known Akt targets. We are currently assessing whether PDK1, a major kinase upstream of Akt which also directly binds PI(3,4)P2, is regulated by INPP4A. Interestingly, other kinases known to interact with PDK1 (PKCdelta, RSK) or to be directly phosphorylated by PDK1 (PKCgamma) showed decreased phosphorylation in cells expressing active INPP4A. We are currently testing the hypothesis that PDK1/Akt and Btk/PLCg2 signalosomes are differentially regulated by INPP4A.

CS24.02

CONCURRENT SESSION 24 – RAPID FIRE PRESENTATIONS - INFLAMMATORY DISEASE AND ECM
July 21, 2016 10:30 – 12:00

Sodium-Glucose Cotransporter Sglt1 (Slc5a1) Is Present in Various Murine Organs with Sex-Related Expression in Kidneys

Ivana Vrhovac Madunić[1], Davorka Breljak[1], Dean Karaica[1], Hermann Koepsell[2], Ivan Sabolić[1]
[1]Molecular Toxicology Unit, Institute for Medical Research and Occupational Health, Zagreb, Croatia; [2]Institute of Anatomy and Cell Biology, University of Wurzburg, Wurzburg, Germany

Abstract: Novel antidiabetic drugs have been developed aimed to lower blood glucose by inhibiting sodium-D-glucose cotransporter 1 (Sglt1) in intestine and kidneys. In mice, the intestinal Sglt1, localized in the enterocyte brush-border membrane (BBM), is responsible for bulk (>80%) glucose absorption, whereas the transporter in the BBM of renal proximal tubules (PT) contributes to ~3% glucose reabsorption. The presence of Sglt1 in other mammalian organs, which could represent possible targets of the novel inhibitors, is poorly known. Here we compared the expression of *Sglt1* mRNA and its protein in various organs of wild type (WT) and *Sglt1* knockout (KO) mice by quantitative RT-PCR and immunocytochemistry (IC). In WT, but not in KO mice, the *Sglt1* mRNA expression was highest in small intestine; high in seminal vesicles, kidneys and salivary glands; medium in prostate, tongue, eyes and uterus, and small in pancreas, lungs and liver. By IC in WT mice, Sglt1 protein was detected in small intestine (enterocytes, BBM), kidneys (PT, BBM; thick ascending limbs, luminal membrane (LM)), eyes (optical nerves), liver (bile ducts, LM), pancreas (ducts, LM), salivary glands (serous acini and initial ducts, LM), tongue (taste epithelium, plasma membrane), prostate (myoepithelial cells), bulbourethral gland (duct cells, LM), seminal vesicles (myoepithelial cells), and uterus (endometrial cells, LM). In kidneys, we observed sex-dependent expression of *Sglt1* mRNA (females>males) and its protein (males>females) indicating different transcriptional and post-translational regulations. Diverse localizations of Sglt1 in various organs may represent targets of the novel inhibitors with unpredictable health consequences in diabetic patients. Funded by Croatian Science Foundation-project#1481.

CS24.03

CONCURRENT SESSION 24 – RAPID FIRE PRESENTATIONS - INFLAMMATORY DISEASE AND ECM
July 21, 2016 10:30 – 12:00

CpG Oligonucleotides Activate Signaling Pathways in Human Neutrophils

Galina Viryasova, Galina Sud'Ina, Ekaterina Golenkina
Belozersky Research Institute of Physico-chemical Biology, Lomonosov Moscow State University, Moscow, Russian Federation

Abstract: Polymorphonuclear leukocytes (PMNLs, neutrophils) play a major role in the initiation and resolution of the inflammatory response. Unmethylated cytosine-phosphate-guanosine (CpG) sequences are present in large amounts in the bacterial DNA and are not typical for mammalian DNA. These structures are recognized by the immune system and may cause a specific immune response, for example, by activating TLR9 and increasing production of cytokines. Synthetic CpG-oligonucleotides (CpG-ODN) in the future may be a component of drugs. We examined the effects of ODN2216 structures, (5'- ggG GGA CGA TCG TCg ggg gg-3 ') containing various amounts of phosphodiester and phosphothioate bonds on neutrophil signaling pathways and cellular responses. Leukotriene B4 (LTB4) and 5-HETE are lipid mediators derived from arachidonic acid via the 5-lipoxygenase (5-LOX) pathway, and produced by neutrophils. LTB4 modulates TLR9 expression on human neutrophils and enhances responsiveness to TLR2 and TLR4 ligands. In this study, CpG ODN2216 inhibited leukotriene synthesis. We have demonstrated that ODN with phosphorothioate bonds activate NO synthethis and superoxide production, which can lead to the formation of peroxynitrite (ONOO-). We suggest that reactive oxygen species, NO and peroxynitrite are signaling molecules that regulate the activity of 5-LOX and neutrophil apoptosis. Fully phosphothioate structures have a pronounced effect of inducing apoptosis. By increasing of the phosphodiester bonds content, proapoptotic action of CpG ODN is reduced. We also showed that ODN2216 with phosphorothioate bonds increase the ability of neutrophils to adhere. We propose that phosphorothioate modification of ODNs represents a potential mechanism of PMNL activation.

CS24.04

CONCURRENT SESSION 24 – RAPID FIRE PRESENTATIONS - INFLAMMATORY DISEASE AND ECM
July 21, 2016 10:30 – 12:00

Controlling Cell Behaviours Through a Novel Research Platform with Modularity Among Integrin Ligands, Rigidity, and Topology

Wilhelm W. Chen, Eileen Fong
Nanyang Technological University, Singapore, Singapore

Abstract: Three aspects of extracellular matrix (ECM) that can determine cell behaviours, such as proliferation, migration, or differentiation, have gained appreciation in the last decade: physical

properties, biochemical cues, and topographical presentation of adhesive epitopes. This study presents a high-quality, readily available research tool, which is the novel 3D culture hydrogel, with reproducible and independent modularity among these three aspects for elucidating the cell-microenvironment interactions in a reductionist manner. Physicochemical characterizations show agreement with theoretical predictions that the clickable hydrogel could be tailored a wide range of rigidity and spatial configurations, such as platform dimensionality, thickness, cell area, shape, and microscale epitope characteristics. Furthermore, using engineered ECM proteins, the integrin specificity was well-defined by presenting α5β1, α3β1 and α6β4 integrin ligands from recombinant fibronectin or laminin-5. We found that the different integrin-ECM interactions mediated primary keratinocytes motility and their integrin-mediated focal adhesion complexes formation. Cells using α3β1 integrin lost their motility and activities of paxillin recruitment. Profiles of adhesion complexes expression and links between ECM-controlled integrin and integrin-mediated signalling pathways, such as tumour-related PI3K/Akt and Wnt/β-catenin, suggest that integrins are integral nodes in signalling pathways underlying diseases, breast cancer for instance. Decoupling the complexity and incorporate key aspects that traditional 2D Petri dishes cannot achieve, this platform can be easily used to study and control cell behaviours. Since α3β1, α5β1 and α6β4 integrin ligands are important biochemical cues in the epithelial basement membrane zone, the work has profound implications for future exploratory studies of epithelial biology and carcinomas.

CONCURRENT SESSION 25 | CS25
NEURODEGENERATIVE DISEASE

CS25.01

CONCURRENT SESSION 25: NEURODEGENERATIVE DISEASE
July 21, 2016 13:30 – 15:00

Mechanisms Operated by Caffeine and Adenosine A2A Receptors to Control Synaptic Plasticity and Neurodegeneration

Rogrigo Cunha
Universidade de Coimbra, Coimbra, Portugal

Abstract: Neurodegenerative Disease

CS25.02

CONCURRENT SESSION 25: NEURODEGENERATIVE DISEASE
July 21, 2016 13:30 – 15:00

Cholesterol Homeostasis in the Brain: Link to the Alzheimer's Disease

Aleksandra Mladenovic Djordjevic[1], Kosara Smiljanic[1], Stjepko Cermak[2], Silva Katusic Hecimovic[2], Selma Kanazir[1]
[1]*Institute for Biological Research "Sinisa Stankovic", Belgrade, Serbia;*
[2]*Rudjer Boskovic Institute, Zagreb, Croatia*

Abstract: Cholesterol plays an essential role in the brain and its content must be precisely maintained in order to keep brain function well. Cholesterol complex homeostasis regulation is still poorly understood but it is known that is critically challenged in the aging brain and disturbed in several of neurodegenerative disease such as Alzheimer's disease (AD). In order to design strategies to treat disturbed cholesterol homeostasis we investigated different forms of dietary restriction (DR), as dietary intake greatly affects brain and neurological health. We showed that DR attenuate age-related changes in the expression of genes that are key regulators of cholesterol metabolism (Mladenovic Djordjevic et al., 2009, Biogerontology 10:735-745) in the same time leaving cholesterol content unchanged (Smiljanic et al., 2013, Lipids 48:1069-1077; 2014, AGE 36:1303–1314.). We have also studied a more direct molecular link(s) between cholesterol homeostasis, membrane trafficking and the metabolism of APP – a key protein in AD pathogenesis. Our hypothesis is that APP C-terminal fragment CTFβ plays an important role in membrane trafficking and altered cholesterol homeostasis. To this end we have used various in vitro cellular models pharmacologically modulated for cholesterol accumulation, lysosomal dysfunction and accumulation of CTFβ fragment. I'll discuss data showing that overexpression of APP significantly influences cholesterol homeostasis in the cell, affecting mRNA and protein expression.

CS25.03

CONCURRENT SESSION 25: NEURODEGENERATIVE DISEASE
July 21, 2016 13:30 – 15:00

Elevated Amyloid Clearance Activity during Transition to Dementia Triggers Increased Levels of Abeta34

Gerhard Multhaup, Filip Liebsch
Pharmacology, McGill, Montreal, Canada

Abstract: Due to its long "preclinical" phase, Alzheimer disease (AD) is traditionally diagnosed during late symptomatic stages when opportunities for causal therapeutic interventions are limited. The vast majority of AD cases are "sporadic" and characterized by the impaired clearance of neurotoxic amyloid beta (Aβ) peptides. We have recently reported that two processing routes exist with stepwise proteolytic cleavages beginning with APP fragments of 49, 51 or 52 residues and both eventuating in Aβ34 (Olsson et al., 2014). The two pathways generate Aβ40 and Aβ42 that are especially prevalent in brain and CSF. Alterations in the degradation process that increase Aβ42 (hence, decrease the Aβ40/42 ratio) are thought to be critical for AD pathogenesis since Aβ42 is markedly amyloidogenic and is believed to be the causative agent. Based on our biochemical data, Aβ34 can be generated from different parent molecules by two not mutually exclusive mechanisms, (i) through the gamma-secretase mediated processing of C99 (beta-stub of APP) and (ii) by BACE1 cleavage of Aβ40 and Aβ42. We found that the C-terminally truncated isoform Aβ34 is an important intermediate product of BACE1-mediated Aβ clearance. Notably, our testing of human clinical samples has revealed Aβ34 significantly elevated in patients with mild cognitive

impairment compared to healthy individuals. We strongly believe that Aβ34 is a novel marker of Aβ clearance activity and we predict that changes in Aβ34 levels in relation to the classical fluid and imaging biomarkers and cognitive performance are an innovative way to predict an individual's conversion from healthy aging to AD.

CS25.04

CONCURRENT SESSION 25: NEURODEGENERATIVE DISEASE
July 21, 2016 13:30 – 15:00

Aurora a Regulation of Spindle-Associated WD40-Repeat Protein 62 is Required for Cell Cycle Progression and Brain Growth

Dominic C.H. Ng[1], Nicholas R. Lim[2], Yvonne Yeap[1], Leonie Quinn[3]
[1]School of Biomedical Sciences, University of Queensland, St Lucia, QLD, Australia; [2]Biochemistry and Molecular Biology, University of Melbourne, Parkville, VIC, Australia; [3]Anatomy and Neuroscience, University of Melbourne, Parkville, VIC, Australia

Abstract: The identification of genetic mutations causative for primary microcephaly (reduced brain size) has informed on the molecular determinants of neuroprogenitor cell proliferation and differentiation required for normal brain growth. The most commonly mutated microcephaly genes encode spindle pole proteins including WD40-Repeat Protein 62 (WDR62), a signaling scaffold protein that binds c-Jun N-terminal kinases (JNKs). Deletion of WDR62 in mice or orthologous genes in Drosophila and Zebrafish recapitulate the microcephaly phenotype indicating a highly conserved neurodevelopmental role. However, the precise molecular and cellular functions of WDR62 remain undefined. Here we reveal that spatiotemporal regulation of WDR62/JNK by centrosomal Aurora A is required for the cell cycle progression and timely division of neuroprogenitor cells in Drosophila and mice. WDR62 is rapidly mobilized from the cytoplasm to bind spindle microtubules specifically during prometaphase. We show that Aurora A activity recruits WDR62/JNK to the spindle pole during this time and this is critical for spindle orientation, mitotic progression and self-renewal of neuroprogenitors. WDR62 microcephaly mutants fail to localize to mitotic spindles, are refractory to AURKA signalling and do not rescue neuroprogenitor division defects in our *in vivo* models which suggest that defects in AURKA/WDR62 signaling may underlie human brain growth deficiencies. In defining the constituents of the mitotic phosphorylation network regulated by the AURKA/WDR62/JNK signalling node, we utilized proximity-labelling mass spectrometry and quantitative phosphoproteomics with stable-isotope labelling to identify novel centrosomal and spindle-regulatory factors as mitotic targets. Thus, our study has defined WDR62-regulated signalling required for spindle regulation and embryonic brain growth.

CONCURRENT SESSION 26 | CS26
POST-TRANSLATIONAL MODIFICATIONS

CS26.01

CONCURRENT SESSION 26: POST-TRANSLATIONAL MODIFICATIONS
July 21, 2016 13:30 – 15:00

Phosphotyrosine Profiling

Shawn Li
University of Western Ontario, London, ON, Canada

Abstract: Phosphotyrosine Profiling

CS26.02

CONCURRENT SESSION 26: POST-TRANSLATIONAL MODIFICATIONS
July 21, 2016 13:30 – 15:00

Genetically Encoded Protein Phosphorylation

Patrick O'Donoghue
Western University, London, ON, Canada

Abstract: Synthetic Biology & Post-Translational Modifications

CS26.03

CONCURRENT SESSION 26: POST-TRANSLATIONAL MODIFICATIONS
July 21, 2016 13:30 – 15:00

Clearance of TDP-43 Pathology Prevents Disease Progression in New Transgenic Mouse Models of ALS and Frontotemporal Dementia

Adam K. Walker[1], Krista J. Spiller[2], Guanghui Ge[2], Allen Zheng[2], Yan Xu[2], Melissa Zhou[2], Kalyan Tripathy[2], Linda K. Kwong[2], John Trojanowski[2], Virginia Lee[2]
[1]Biomedical Sciences, Macquarie University, Sydney, Australia; [2]Center for Neurodegenerative Disease Research, University of Pennsylvania, Philadelphia, PA, United States of America

Abstract: Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are incurable neurodegenerative diseases defined by accumulation of pathology containing the RNA/DNA-binding protein TDP-43. In disease, TDP-43 is cleared from the nucleus and is post-translationally modified by phosphorylation, ubiquitination and C-terminal cleavage, forming cytoplasmic detergent-insoluble aggregates in neurons. To analyse the effects of cytoplasmic TDP-43, we constructed new transgenic mice that inducibly expressed this protein in brain and spinal cord. TDP-43 solubility, post-translational modification, neuron loss and muscle innervation were analysed, with motor behaviour testing to investigate disease progression. Expression of cytoplasmic TDP-43 under the control of the *neurofilament heavy chain* (*NEFH*) promoter resulted in the

first mouse model to recapitulate both pathological and behavioural features of ALS: these mice accumulated detergent-insoluble pathological cytoplasmic TDP-43 in the brain and spinal cord, accompanied by progressive motor dysfunction, brain and muscle atrophy, neuromuscular junction denervation, neurodegeneration, weight loss and decreased survival. Remarkably, when cytoplasmic TDP-43 expression was halted after neurodegeneration had begun and motor phenotype was severe, the mice completely cleared the pathology, nuclear TDP-43 returned, neuron loss was halted, muscle was reinnervated and normal movement returned with dramatic extension of life span. These findings indicate that amelioration of TDP-43 pathology prevents disease progression even at late disease stages, providing hope that pharmacologially targeting TDP-43 accumulation could be beneficial for patients even after symptom onset and deterioration has occurred.

CS26.04

CONCURRENT SESSION 26: POST-TRANSLATIONAL MODIFICATIONS
July 21, 2016 13:30 - 15:00

Proteogenomic Analyses of Breast Cancer Cells Reveal Oncogenic Properties of CDK12 from Misregulation of Alternative Splicing

Jerry F. Tien, Alborz Mazloomian, S.-W. Grace Cheng, Christopher S. Hughes, Christalle C.t. Chow, Leanna Canapi, Arusha Oloumi, Genny Trigo-Gonzalez, Vicky C.-. Chang, Stella S. Chun, Sohrab P. Shah, Samuel Aparicio, Gregg B. Morin
BC Cancer Agency, Vancouver, BC, Canada

Abstract: CDK12 (cyclin-dependent kinase 12) is a regulatory kinase with evolutionarily conserved roles in modulating transcription elongation. Recent tumour genome studies of breast and ovarian cancers revealed recurrent alterations in the *CDK12* gene. Loss of CDK12 function disrupts DNA repair pathways, while preliminary evidence suggests that genomic amplification of *CDK12* is associated with increased tumourigenicity of cancer cell lines. *CDK12* therefore possesses characteristics of tumour suppressor genes and possibly oncogenes; however, the molecular mechanisms underlying its function are poorly defined. Based on global analysis of RNA transcripts in normal and breast cancer cell lines, we demonstrate here that CDK12 primarily regulates a specialized subtype of alternative mRNA splicing: alternative last exons (ALEs). While the affected genes are highly cell type-specific, they generally feature long transcripts, suggesting a common mode of regulation. ALE splicing represents a significant function of CDK12, as it can be used to modulate the expression of *ATM*, a key component of DNA repair. CDK12 also regulates ALE splicing of *DNAJB6*, and increases the formation of a DNAJB6 isoform previously found to influence tumour cell invasion. Consistent with that finding, we show that CDK12 promotes the invasiveness of breast tumour cells in a dose-dependent manner. These findings suggest that over-expression of CDK12, as often seen in *HER2*-positive breast tumours, can be a contributing factor to the pathogenesis of the cancer. Together, these results demonstrate how genomic alterations in *CDK12* can affect the tumourigenicity of breast cancer cells through the mis-regulation of alternative splicing.

CONCURRENT SESSION 27 | CS27
MEMBRANE PROTEINS

CS27.01

CONCURRENT SESSION 27: MEMBRANE PROTEINS
July 21, 2016 13:30 - 15:00

The Other Synapse: Calcium-Dependent Exocytosis in the Immune System

Jens Rettig
Universität des Saarlandes, Saarland, AB, Germany

Abstract: Secretory Vesicles & Ion Channels

CS27.02

CONCURRENT SESSION 27: MEMBRANE PROTEINS
07-21-2016 13:30 - 15:00

Ligand-Induced CD36 Receptor Nanoclusters Growth and Compaction Promote Signal Transduction

John M. Githaka[1], Anthony Vega[2], Michelle A. Baird[3], Michael W. Davidson[3], Khuloud Jaqaman[2], Nicolas Touret[1]
[1]Biochemistry, University of Alberta, Edmonton, AB, Canada; [2]Biophysics, UT Southwestern Medical Center, Dallas, TX, United States of America; [3]National High Magnetic Field Laboratory and Department of Biological Science, Florida State University, Tallahassee, FL, United States of America

Abstract: Nanoclustering is emerging as a key organizational principle of membrane proteins and supporting the signal transduction efficacy of receptors. Using super-resolution imaging, we investigated the molecular organization of the clustering-responsive receptor CD36 and its downstream effector Fyn in response to thrombospondin-1 (TSP-1), an anti-angiogenic ligand that promotes endothelial cells apoptosis. At steady state, CD36 receptors pre-exist in nanoclusters (diameter of about 100 nm). Fyn is already present in these clusters, and its N-terminal membrane targeting domain is sufficient for this association. Even if already associated, we found that Fyn only becomes activated activated upon TSP-1 binding through an enhancement of CD36-Fyn clustering, forming larger and denser clusters. These enhancements are supported by the actin cytoskeleton and the presence of plasma membrane cholesterol as their perturbation abolishes signaling. Our data demonstrate cooperation between cholesterol-dependent domains and the cortical actin cytoskeleton in the reorganization of the receptor-effector pair CD36-Fyn that enables signaling during TSP-1 stimulation.

CS27.03

CONCURRENT SESSION 27: MEMBRANE PROTEINS
July 21, 2016 13:30 – 15:00

Engulfment During Phagocytosis: Combining Modelling and Experiment

David Richards
Exeter University, Exeter, United Kingdom

Abstract: Despite being of vital importance to the immune system, the mechanism by which cells engulf relatively large solid particles during phagocytosis is still poorly understood. I will discuss my recent work on the rate of engulfment during phagocytosis, which combines mathematical modelling with dual-micropipette time-lapse images of neutrophil phagocytosis. This shows that engulfment is actually a two-stage process, with an initially slow diffusive stage followed by a much quicker, probably actively driven second stage. I will then discuss the role of target shape and orientation during phagocytosis, covering various shapes such as spheres, ellipsoids, capped-cylinders and hourglasses. This has direct application to both nanoparticle drug delivery and how some bacteria avoid being internalised simply by their shape. Finally, I will demonstrate how the modelling explains why non-spherical particles tend to engulf quickest when presented to the cell tip-first.

CS27.04

CONCURRENT SESSION 27: MEMBRANE PROTEINS
July 21, 2016 13:30 – 15:00

Structure-Function Correlation in the Apelinergic System

David N. Langelaan[1], Kyungsoo Shin[1], Aditya Pandey[1], Muzaddid Sarker[1], Shuya K. Huang[1], Danielle Leblanc[1], Nigel A.i. Chapman[1], Xiang-Qin Liu[1], Denis J. Dupré[2], Jan K. Rainey[1]
[1]Biochemistry & Molecular Biology, Dalhousie University, Halifax, NS, Canada; [2]Pharmacology, Dalhousie University, Halifax, NS, Canada

Abstract: The apelinergic system comprises a class A G-protein coupled receptor (GPCR), the apelin receptor (AR), and two cognate peptidic ligands, apelin and apela. Apelin was discovered in 1998 and relatively widely studied; apela, conversely, was first reported in late 2013. The apelinergic system initially acts during cardiovascular system development, through apela-AR activation. Following development, apelin appears to take over as the primary cognate ligand for AR, although apela mRNA expression was recently detected in adult rodent heart tissue. Apelin has physiological roles in regulating cardiovascular system function; the adipoinsular axis; and, the brain and central nervous systems. It is also a highly potent inotrope, functionality now also shown for apela. From a pathological standpoint, the apelinergic system is hijacked in tumour neoangiogenesis and has been associated with chronic changes in blood pressure; apelin levels are directly elevated in correlation to body-mass index; and, AR is a CD4-coreceptor for HIV1 infection. Apelin and apela are unusual peptide hormones, in that each has multiple bioactive isoforms. Isoform length modulates downstream apelin-AR signalling; apela likely behaves similarly, but this has not yet been shown. Using nuclear magnetic resonance (NMR) spectroscopy, our group has provided the only high-resolution structural data for the apelinergic system to date. Structural features of apelin have informed rational design of bioactive analogues; those of the AR have directly added to our understanding of its function. This presentation will detail our current understanding of structure-function correlation in the apelinergic system alongside the challenges encountered in reaching this point.

CONCURRENT SESSION 28 | CS28
CANCER STEM CELLS

CS28.01

CONCURRENT SESSION 28: CANCER STEM CELLS
July 21, 2016 13:30 – 15:00

Dissecting the Role of the Wnt and Other Signaling Pathways in Liver Development and Disease

Jim Woodgett
University of Toronto, Toronto, ON, Canada

Abstract: Signaling and Differentiation in Stem/Cancer Cells

CS28.02

CONCURRENT SESSION 28: CANCER STEM CELLS
July 21, 2016 13:30 – 15:00

Controlling the Balance of Self-Renewal and Differentiation in Human Brain Tumors

Peter Dirks
Hospital for Sick Children, Toronto, ON, Canada

Abstract: Brain Cancer Stem Cells

CS28.03

CONCURRENT SESSION 28: CANCER STEM CELLS
July 21, 2016 13:30 – 15:00

Comprehensive Sequence Analysis of Relapse & Refractory Pediatric Acute Myeloid Leukemia

Emilia Lim[1], Diane Trinh[2], Rhonda Ries[3], Jim Wang[4], Yussanne Ma[1], James Topham[1], Maya Hughes[3], Erin Pleasance[1], Andrew Mungall[1], Richard Moore[1], Yongjun Zhao[1], E. Anders Kolb[4], Alan Gamis[5], Malcolm Smith[6], Daniela Gerhard[7], Robert Arceci[8], Todd Alonzo[5], Soheil Meshinchi[3], Marco Marra[1]
[1]BC Cancer Agency - Genome Sciences Center, Vancouver, Canada; [2]BC Cancer Agency - Genome Sciences Centre, Vancouver, BC,

Canada; [3]Fred Hutchinson Cancer Research Center, Seattle, WA, United States of America; [4]Children's Oncology Group, Arcadia, CA, United States of America; [5]Children's Oncology Group, Arcadia, United States of America; [6]Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, MD, United States of America; [7]Office of Cancer Genomics, National Cancer Institute, Bethesda, MD, United States of America; [8]Phoenix Children's Hospital, Phoenix, AZ, United States of America

Abstract: Introduction: Induction chemotherapy results in complete remission in 80% of children with acute myeloid leukemia (AML). However, many patients either fail to achieve a remission, or relapse after an initial response and subsequently die of their disease. **Method:** To identify prognostic markers and therapeutic targets, we provide a sequence-based characterization of the pediatric AML transcriptome. We performed miRNA-seq on 637 primary, 22 refractory and 37 relapse samples, and mRNA-seq on 177 primary, 12 refractory and 47 relapse samples. **Result(s):** Cox proportional hazards analyses identified miRNAs (including members of the miR-106a-363 cluster) that were associated with inferior overall survival (OS) and event free survival (EFS) (Hazards Ratio: 1.36-2.14; q-value<0.05). In addition, miR-106a-363 was abundantly expressed in relapse and refractory samples and in primary samples of refractory patients. Integrative miRNA:mRNA analyses further indicated that several candidate targets of miR-106a-5p are involved in oxidative phosphorylation, a process that is suppressed in treatment-resistant leukemic cells. **Conclusion(s):** Through a detailed analysis of the transcriptome, we identified miRNAs whose expression levels were significantly associated with clinical outcome. In addition, we showed that abundant expression of miR-106a-5p might contribute to treatment resistance by modulating genes involved in energy metabolism. **Outcome/Impact:** Overall, our transcriptome profiles provide clinically meaningful data for risk and response identification and define novel pathways that may be amenable to therapeutic targeting.

CS28.04

CONCURRENT SESSION 28: CANCER STEM CELLS
July 21, 2016 13:30 – 15:00

Heterogeneous Nuclear Ribonucleoprotein L (hnRNP L) Controls Caspase-Dependent Cell Death in Hematopoietic Stem Cells

Anne Helness[1], Charles Vadnais[2], Jennifer Fraszczak[2], Tarik Moroy[2]
[1]Institut de recherches cliniques de Montreal - IRCM, Montreal, AB, Canada; [2]Institut de recherches cliniques de Montreal - IRCM, Montreal, Canada

Abstract: The proliferation and survival of hematopoietic stem cells (HSCs) has to be strictly coordinated to ensure the timely production of all blood cells. Here we report that the splice factor and RNA binding protein hnRNP L (heterogeneous nuclear ribonucleoprotein L) is required for hematopoiesis, since its genetic ablation in mice reduces almost all blood cell lineages and causes premature death of the animals. In agreement with this, we observed that hnRNP L deficient HSCs lack both the ability to self-renew and foster hematopoietic differentiation in transplanted hosts and show high levels of AnnexinV binding suggesting that they undergo apoptosis. HSCs lacking hnRNP L also display mitochondrial dysfunction and elevated levels of reactive oxygen species (ROS). Moreover, lin$^-$c-Kit$^+$ fetal liver cells from hnRNP L deficient mice show high p53 protein levels and up-regulation of p53 target genes indicating an ongoing DNA damage response possibly caused by elevated ROS levels. Finally, cells lacking hnRNP L also up-regulated the expression of the death receptors *TrailR2* and *CD95/Fas* and show Caspase-3, Caspase-8 and Parp cleavage. Interestingly, neither the overexpression of Bcl-2, which could counteract mitochondrial dysfunction nor deletion of p53, which is required for DNA damage induced cell death restored viability in hnRNP L deficient HSCs. However, treatment with the pan-caspase inhibitor Z-VAD-fmk, restored cell survival in hnRNP L deficient cells. These data suggest that hnRNP L is critical for the survival and functional integrity of HSCs by restricting the activation of caspase-dependent death receptor pathways through several mechanisms.

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POSTERS

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July 18 & 19 2016	**10:00 – 18:45**
July 20, 2016	**10:00 – 13:30**

Presenters will be available to answer questions during the networking breaks.

PP01.01

Metabolic Stress Responses in Multi-Tissue of Goldfish under Glyphosate Exposure and Underlying Mechanisms

Minghui Li, Junsong Wang
Nanjing University of Science and Technology, Nanjing, China

Abstract: Glyphosate as one of the most extensively used herbicide worldwide, its toxicity to non-target organisms is indeterminate. In this study, goldfish (Carassius auratus) were exposed to different concentrations of a glyphosate based herbicide for 96 hours. Plasma was collected and the hematological parameters of GOT, GPT, LDH, BUN and CRE were quantified. Tissues of brain, kidney and liver were collected and submitted to histopathological inspection and NMR-based metabolomics analysis. Exposure to glyphosate produced increases in hematological parameters of BUN and CRE tested. Histopathological inspection revealed tissue-specific and dose-dependent injuries under glyphosate stress. Metabolomics analysis revealed significant perturbations in the neurotransmitter equilibrium, energy metabolism, amino acids metabolism as well as intestinal microbial metabolism in glyphosate dosed fish, which were associated with the toxicity of glyphosate. 1H NMR-based metabolomics approach was proved to be an effective tool for the global perspective of the metabolic responses of non-target organisms to pesticides and it is suitable for the inspection of the environmental risks of pesticides and the explanation of the underlying mechanisms.

PP01.02

Cancer Up-Regulated Gene (CUG)2, a Novel Oncogene Induces Epithelial-Mesenchymal Transition via TGF-Beta

Sirichat Kaowinn, Chuanpit Ruangcharus, Young-Hwa Chung
Cogno-mechatronics Engineering, Pusan National University, Busan, Korea, Republic of

Abstract: CUG2, a novel oncogene has been involved in cell migration and invasion, but the underlying mechanism on CUG2-mediated oncogenesis has not revealed. In this study, CUG2 decreased levels of E-cadherin protein and increased expression of N-cadherin and vimentin, representative characteristics of epithelial-mesenchymal transition (EMT). A CUG2 deletion mutant, lack of interaction with NPM1 failed to induce EMT. Furthermore, CUG2 enhanced expression of Snail and Twist, and activation of Smad2/3, downstream molecules of TGF-β signaling while the CUG2 mutant did not, indicating that CUG2-mediated EMT requires NPM1 interaction. Of interest, TGF-β conversely elevated CUG2 expression via a close cooperation of Sp1 and Smad2/3, indicating crosstalk of CUG2 and TGF- β signaling. Treatment with LY364947, an inhibitor of TGF- β signaling drastically reduced CUG2-mediated EMT, leading to decrease of wound healing and cell invasion. Taken together, we suggest that CUG2 induces EMT of human lung cancer cells via TGF- β signaling.

PP01.03

Cancer Upregulated Gene (CUG) 2, a Novel Oncogene, Induces Cancer Stem Cell-Like Phenotypes via TGF-Beta Signaling

Sirichat Kaowinn, Chuanpit Ruangcharus, Young-Hwa Chung
Cogno-mechatronics Engineering, Pusan National University, Busan, Korea, Republic of

Abstract: Cancer stem cell (CSC)-like phenotype is closely related to epithelial-mesenchymal transition (EMT). Since we have observed that CUG2 induces EMT, we hypothesize that CUG2 enables to induce CSC-like phenotypes. To test our hypothesis, we first explored whether transcription factors related to stemness are upregulated in CUG2-overexpressing lung cancer cells. Expression of BMI1, Oct4 Sox2, Klf4, and Nanog expression was enhanced compared to those in control cells. Consequently, CUG2 increased sphere formation and activity of alkaline phosphatase. Introduction of a CUG2 mutant, lack of NPM1 failed to induce CSC-like phenotypes. Additionally, suppression of NPM1 with its siRNA resulted in the same results, indicating that NPM1 is required for CUG2-mediated CSC-like phenotypes. When we wondered whether TGF-β signaling is also involved in CUG2-mediated CSC-like phenotypes, Smad2, or 3 expression was inhibited by its siRNA. We then found that reduction of Smad 2 or 3 expression results in lower levels of transcription factors related to stemness. Inhibition of JNK and p38 MAPK, non-canonical TGF-β signaling molecules, also reduced the expression of transcription factors related to stemness. Finally, when we suppressed each transcription factor with its siRNA, we examined sphere formation and activity of alkaline phosphatase. Decrease of Nanog expression still induced CSC-like phenotypes while the others failed, suggesting that Nanog did not play a crucial role in this situation at least. Taken together, we suggest that CUG2 induces CSC-like phenotypes through TGF-β signaling.

PP01.04

Radical Scavenging Effect of Aqueous or Ethanol Leaf Extracts of S.glauca DC (Paradise Tree)

Sammy Davies E. Osagie-Eweka, Jerry N. Orhue, Diamond O. Ekhaguosa
Biochemistry, University of Benin, Benin, Nigeria

Abstract: Aims: The study was conducted to evaluate the phytochemical and antioxidant potentials of ethanol and aqueous leaf extracts of Simarouba glauca vis-à-vis standard antioxidants. Methodology: Samples was harvested, air dried, pulverized and

extracted with aqueous and absolute ethanol; freeze dried at the National energy commission centre, University of Benin. Phytochemical and antioxidant studies was conducted based on already extablished methods and principles.Results: DPPH radical scavenging activity yielded aqueous and ethanol extracts IC50 values of 3.2144 and 4.9100 ×g/ml respectively. Reducing power activity yielded (aqueous and ethanol extracts) EC50 of values 60.3233 and 60.1000 ×g/ml respectively. Total antioxidant activity yielded (ethanol and aqueous extracts) IC50 values of 52.4320 and 68.8201 ×g/ml respectively. Hydroxyl radical activity yielded (ethanol and aqueous extracts) IC50 values of 49.3130 and 50.2341 ×g/ml respectively. Trolox equivalent antioxidant activity yielded (ethanol and aqueous extracts) IC50 values of 45.2015 and 52.0721 ×g/ml respectively. Nitric oxide scavenging activity yielded aqueous IC50 value of 14.2102 ×g/ml but ethanol extract yielded no inhibition concentration at 50 percent. Conclusion: The study showed that aqueous and ethanol leaf extracts of S. glauca demonstrated substantial amount of biochemically valuable phytochemicals and antioxidant potential capable of scavenging reactive oxygen species.

PP01.05

Molecular Characterization of Some Equine Exotic Viruses in Saudi Arabia

Mohammed A. Al-Hammadi[1], Abdelmohsen A. Alnaeem[1], Maged G. Hemida[2]
[1]Microbiology and Parasitology, King Faisal University, Al-Hufuf, Saudi Arabia; [2]Microbiology and Parasitology, King Faisal University, Alahsa, Saudi Arabia

Abstract: Equine Exotic viruses (Equine Influenza (EIV), West Nile (WNV), Equine Arteritis (EAV), and African Horse sickness (AHSV)) continue to pose great risks to Equine Industry worldwide. Despite vaccine application of some of these viruses, many outbreaks still report globally. Little is known about the molecular characterization of these viruses in Saudi. The major goals of the current study were to detect the presence of nucleic acids (NAs) of these viruses, to evaluate the immune status of different horses population across the kingdom. To achieve our goals, nasal swabs, rectal, swabs and sera were collected from 250 animals across the kingdom. Detection of the viral NAs was done by commercial Real Time PCR kits while detection of the antibodies against these viruses by the commercial ELISA kits. Our data clearly showing detection of EIV (19.2%), WNV (22.8), and EAV (19.9%) in several horses population in Saudi Arabia using Real Time PCR technique. Furthermore, the high seroprevalence of EIV, WNV, and EAV in the tested sera was reported. Meanwhile, we failed to detect both the viral NAs and antibodies in swabs and sera of tested animals against AHSV. In conclusion, this study will pave the way for further molecular characterization of these exotic viruses in the Kingdome. To the best of our knowledge, this is the first study dealing with molecular based prevalence of EIV, WNV, EAV and AHSV across the Kingdom. This work has been funded by the King Abdul Aziz City of Science and Technology grant No (ARP-34- 117).

PP01.06

PPAR/NF-kB – Dependent Mechanism of N-Stearoylethanolamine Anti-Inflammatory Action

Oleksandra Onopchenko, Andrey Berdyshev, Halyna Kosiakova, Nadiya Hula
Palladin Institute of Biochemistry of the National Academy of Sciences of Ukraine, Kyiv, Ukraine

Abstract: N-stearoylethanolamine (NSE) – is saturated minor compound of natural origin that represents the large family of signaling lipids N-acylethanolamines, which belong to endocannabinoid system. Considering the crosstalk between low-grade chronic inflammation to most spreading metabolic diseases, particularly diabetes, our studies are aimed to investigate the mechanism of anti-inflammatory action of NSE. Earlier we found that NSE reduces serum level of main pro-inflammatory cytokines in rats with obesity-induced insulin resistance. Further *in vitro* investigations showed that administration of NSE to the culture medium at concentration of 10^{-7} M reduces by 60-80% the NF-κB translocation to the nucleus of LPS-activated peritoneal macrophages, which were obtained from normal rats. This finding correlated with the downregulation of IL-1β production (r = 0.87). Moreover, the results of flow cytometry analysis showed the prevention of reactive oxygen species (superoxide radical and hydrogen peroxide) formation under NSE action in LPS-activated peritoneal macrophages. We proposed that this NSE effect may be realized via activation of nuclear receptors – PPARs. *In vitro* studies, using synthetic selective activator of PPARα/γ receptors LY-171,883, selective inhibitors of PPARγ – GW9662 and PPARα – GW6471, confirmed that NSE implements its biological action primarily via PPARγ. In addition, the molecular docking analysis revealed that the highest affinity of NSE is to PPARγ. Importantly, the comparative docking of stearic acid and NSE evaluated that the main role in NSE binding to PPAR ligand-binding domain played the ethanolamine residue. Therefore, the present studies indicate previously unknown PPAR/NF-κB – dependent pathway of anti-inflammatory action of NSE.

PP01.07

An Alcoholic Fatty Liver Disease Model in Zebrafish (Danio Rerio) Optimized by Using Ccd-Rsm Method

Minghui Li, Junsong Wang
Nanjing University of Science and Technology, Nanjing, China

Abstract: Context: Pathological mechanism of alcoholic fatty liver disease (AFLD) caused by excessive alcohol consumption is still unclear. Currently, there are no optimized AFLD models on zebrafish. Objective: The present study seeks to develop a zebrafish model of AFLD by using central composite design-response surface methodology (CCD-RSM) method. Materials and method: AFLD was induced in zebrafish by repeated immersing zebrafish in normal and alcoholic solution alternately, various parameters such as alcohol concentration, exposure hours and exposure days were optimized by using CCD-RSM method. Histopathological inspection was used to detect the liver injury. Results: The optimized conditions for the AFLD model were as

following: exposing zebrafish to 1.17% alcohol for 7.68 hours per day and lasting for 2 weeks. Under these conditions, significantly increased fatty vacuole in liver of zebrafish was found. Discussion and conclusion: The AFLD model on zebrafish was successfully established, which should be helpful for drugs screening and for excavation of the molecular mechanisms of AFLD.

PP01.08

Altered Cholesterol Level and Synaptic Vesicle Fusion Implicated in Impaired Neurotransmission under Vitamin D3-Deficiency

Ludmila A. Kasatkina, Irene O. Trikash
The Department of Neurochemistry, Palladin Institute of Biochemistry, Kyiv, Ukraine

Abstract: 1,25-Dihydroxycholecalciferol, an active metabolite of vitamin D_3, not only impacts Ca^{2+} and phosphorus homeostasis, but also regulates lipid metabolism, balanced immune response, myelination after nerve injury and retardation of age-related cognitive decline. The aim of this work was to characterize the glutamate secretion in cortical nerve terminals and the synaptic vesicle fusion competence in experimental model of alimentary vitamin D-deficiency. Rats were kept on diet (60 days) without cholecalciferol or supplemented with 40 IU of cholecalciferol/100 g of body weight per day. Vitamin D-deficiency was associated with the decreased serum 25-OH-D_3 level (100.642.2 nM vs 38.341.2 nM); the cholesterol level in synaptic plasma membranes increased by 27% which strongly correlated with higher cholesterol level in synaptic vesicle membranes. The survival of primary cortical neurons was slightly decreased and when normalized for viable cells in both groups, primary neurons from vitamin D-deficient rats displayed attenuated exocytotic peak and glutamate release compared to neurons of control animals. The depolarization-induced secretion of pH-sensitive probe was attenuated by 18.344.1% which was accompanied by the corresponding reduction of glutamate release from 26.743.2 to 2042.5 nmol of L-glutamate per mg of protein at 5th min (n=7). In cell-free system developed for studies of synaptic vesicles interaction with various target membranes we showed that cytosolic proteins and synaptic plasma membrane are the critical components that undergo changes in vitamin D-deficiency and affect the exocytosis process, while synaptic vesicles of vitamin D-deficient rats did not change their fusion competence.

PP01.09

Germination and Mitochondrial Respiration Rate in Vigna unguiculata (Bean) Seeds Exposed to Crude Oil

Stella O. Olubodun[1], George E. Eriyamremu[2], Sheena E. Omoregie[2], Nekpen Erhunse[2], Tinu O. Okugbo[3]
[1]Medical Biochemistry, University of Benin, BENIN, Nigeria; [2]Biochemistry, University of Benin, BENIN, Nigeria; [3]Basic Sciences, Benson Idahosa University, BENIN, Nigeria

Abstract: *Vigna unguiculata* (bean) seeds were treated with 0%, 2%, 5% and 10% crude oil fractions [whole crude (WC), water soluble fraction (WSF), and water insoluble fraction (WIF)] for 21 days. Crude oil stress in some plant species leads to formation of reactive oxygen specie. However, evidence are lacking on how crude oil stress affects respiration rate of developing *V. unguiculata*. The objective of the study is to determine how the radicles of *V. unguiculata* respond to crude oil stress in growth and respiration rate. Experiments carried out in equal amounts of the developing radicles after 7, 14 and 21 days post germination (DPG) were performed to determine plant height, radicles/roots length and respiration rate. The data obtained were statistically analysed by descriptive statistics and analysis of variance. After 21 DPG, seedling height of *V. unguiculata* in 10% WC, WSF and WIF had 34.9% 46.4% and 47.4% decrease respectively. While radicle lengths of V. unguiculata in 10% WC, WSF and WIF at 7 DPG, had 4.8%, 42.9% and 14.3 decrease respectively, the root lengths had 4.2% WC, 58.3% WSF, and 33.3% WIF decrease. Decreased respiration rate was observed in the roots of *V. unguiculata* in the different crude oil fractions when compared with control but this was not found to be significant ($P<0.05$) with increase in crude oil contamination or increase in DPG. We suggest that reduction in root length and respiration rate of *V. unguiculata* in crude oil stress may be responsible for the observed decrease in growth and development.

PP01.10

Targeted Knockdown of the DJ-1 Protein Leads to Myeloma Cell Death via Upregulation of the KLF6 Signalling Pathway

Prahlad Raninga[1], Giovanna Di Trapani[2], Slavica Vuckovic[3], Kathryn Tonissen[1]
[1]School of Natural Sciences & Eskitis Institute for Drug Discovery, Griffith University, Nathan, Brisbane, QLD, Australia; [2]School of Natural Sciences, Griffith University, Nathan, Brisbane, QLD, Australia; [3]QIMR Berghofer Medical Research Institute, Brisbane, QLD, Australia

Abstract: Multiple myeloma (MM) is an incurable plasma cell malignancy. Introduction of the proteasome-inhibitors, bortezomib and carfilzomib, has improved MM prognosis and survival; however drug resistance remains a major clinical hurdle. Hence, identifying novel signalling pathways involved in myeloma progression may help to design better anti-myeloma therapies. DJ-1, a Parkinson's disease-associated protein also called Park7, is upregulated in many cancers and its inhibition suppresses tumor growth by modulating the Nrf2, PTEN, ASK, and NF-β signalling pathways. However, the cytoprotective role of DJ-1 and underlying mechanism in MM remains unknown. We found increased DJ-1 expression in MM patient cells, which correlated with shorter overall survival and poor prognosis in MM patients. Targeted DJ-1 knockdown using siRNAs induced myeloma cell death and also restored the sensitivity of bortezomib-resistant myeloma cells to bortezomib. KLF6, a tumor suppressor, is under-expressed in many cancers, and increased KLF6 expression induces cell death. However, how the KLF6 signalling pathway is regulated and drives MM progression remains elusive. We found that KLF6 is expressed at lower levels in myeloma patient cells compared to normal PBMCs, and DJ-1 knockdown increased KLF6 expression in myeloma cells. Moreover, DJ-1 downregulation in conjunction with bortezomib significantly

increased KLF6 expression in bortezomib-resistant myeloma cells. Thus, this study identified KLF6 as a downstream effector pathway of DJ-1 that mediates myeloma cell death in response to DJ-1 inhibition. Our findings demonstrate that the DJ-1/KLF6-axis plays a significant role in determining myeloma cell fate and modulating this axis holds a promising therapeutic strategy to treat MM patients.

PP01.11

Testing for Membrane-Modulated Proprotein Processing

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Abstract: In the membrane catalysis theory, a ligand must interact with the membrane prior to its cell surface receptor. Membrane-ligand association is theorized to enhance the rate of receptor binding by increasing local concentration of ligand; reducing diffusion from a 3D to a 2D process; and, inducing conformational change for receptor recognition. This theory may also apply to proprotein-enzyme interactions, since these enzymes may be membrane localized in the secretory pathway or on the cell surface. Variations in membrane composition mean that a proprotein may encounter a variety of environments, for potential lipid-dependent preferences in association and conformational change. To test for this, we employed proapelin and proapela (also called ELABELA/Toddler). Both proproteins can be processed into multiple isoforms, each of which binds to a class A GPCR (the apelin receptor). This, in turn, regulates multiple physiological systems, particularly the cardiovascular system. Far-UV CD and solution-state NMR spectroscopies showed that proapelin exhibits β-turn characteristics in the presence of anionic but not zwitterionic detergent micelle conditions, suggestive of a preferential lipid interaction. Proapela, conversely, showed similar conformational changes in both zwitterionic and anionic micelles. Since membrane compositions vary between cell types, changes in membrane association and the resulting induced conformation may modulate proapelin or proapela processing. To test this hypothesis, proapelin and proapela processing are being studied using established cell-lines expressing proprotein convertases either endogenously or through transfection. Demonstration of membrane-catalyzed differences in proprotein processing would represent an alternate, highly physiologically and therapeutically relevant means to regulate proprotein processing.

PP01.12

Elevated O-GlcNAcylation Levels Improve Mitochondrial Function

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Abstract: O-linked *N*-acetylglucosamine (O-GlcNAc) is a highly dynamic post-translational modification that involves an addition of a single *N*-acetylglucosamine to serine and threonine residues in mitochondria, nuclear and cytoplasmic proteins. O-GlcNAc cycling is the addition and removal of O-GlcNAc by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA) respectively. Disruptions in O-GlcNAc cycling contribute to diseases such as neurodegeneration. Accumulative dysfunctional mitochondria can also lead to the development of neurodegeneration, and importantly, O-GlcNAcylation regulates mitochondrial function. Previously, we found that altered OGT/OGA expression disrupted mitochondrial protein expression. Furthermore, mitochondrial morphology was disorganized with altered cristae and changes in shape and size. Both cellular respiration and glycolysis was impaired in the OGT/OGA overexpressed cells. These data suggest that O-GlcNAc cycling was essential for the proper regulation of mitochondrial function. Therefore, we hypothesized that prolonged elevations in cellular O-GlcNAc levels would alter the metabolic profile of the cell. We disrupted O-GlcNAc cycling by either treating SH-SY5Y neuroblastoma cells with Glucosamine (GlcN) or OGA inhibitor Thiamet-G (TMG). We found that prolonged TMG/GlcN treatment improved mitochondrial function. Reactive oxygen species (ROS) production was significantly reduced and the protein expression of the mitochondrial antioxidant, manganese superoxide dismustase (MnSOD) was lower. Then, ATP production was lower. Furthermore, we found that mitochondrial were longer in shape and mitochondrial fusion/fission protein expressions were decreased. Altogether, these data demonstrate that prolonged alterations to the cellular homeostasis of O-GlcNAc improve mitochondrial function by reprogramming metabolic programs. Further understanding of how O-GlcNAc cycling regulates metabolism will provide new insights into metabolic diseases such as Alzheimer's.

PP01.13

Human Milk Factor Stimulating Fab-Arm Exchange: A New Method of Bispecific Antibody Production

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Abstract: We have shown that human milk contains a factor stimulating the exchange of HL-fragments (Fab arms) between IgG and sIgA molecules. Here we show that the factor is one of major human milk proteins. Based on obtained results, we plan to develop a method for producing bispecific antibodies from natural monospecific molecules. Bispecific immunoglobulin molecules

contain simultaneously two distinct antigen-binding sites. The use of bispecific molecules allows bring together a tumor cell and a cytotoxic T lymphocyte, which significantly increase the chances of natural elimination of degenerated cells. Significant advances in the design of bispecific antibodies in recent years have led to the creation of therapeutic molecules for intravenous and intramuscular administration. The initial barrier of cytokine response was partially overcome by new designs that improve clinical effect and have lower toxicity. Several preparations of bispecific immunoglobulins are successfully tested in clinical trials. There are described several approaches to the production of bispecific antibodies: bispecific immunoglobulins may be directed in all possible combinations against effector cells, medicines, toxins, DNA, enzymes, and various structures on tumor cells. Development of new artificial bispecific immunoglobulins is directed on optimization of tissue penetration, stability *in vivo*, binding specificity and increased affinity for the target tumor cells. The reported study was partially supported by RFBR, research projects 16-34-00163 mol_a, 16-34-60066 mol_a_dk, 16-04-00603 a, 16-04-00604 a.

PP01.14

Synthesis of Fluorescent Nanoparticles by Using Bacteria from Antarctica: New Prospects from Mysterious Inhabitants

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Abstract: Fluorescent nanoparticles or quantum dots (QDs) are inorganic crystalline structures that possess unique size-dependent properties compared to larger scale materials. QDs are extensively considered as highly versatile tools for biological research, particularly in biomedicine. Main problems with traditional synthesis of these nanoparticles are the generation of toxic byproducts, low biocompatibility and high production costs; hence, greener and cheaper methods are needed. Nowadays, by using biological systems, especially prokaryotes, has emerged as a promising alternative for QDs production. Previously, we determined that bacterial biosynthesis is related to factors as cellular redox status and antioxidant defenses. Based on this, the unusual mixture of extreme environmental conditions of Antarctica would allow the development of natural QDs producing bacteria. In this work, we isolated and characterized cadmium and tellurite resistant Antarctic bacteria capable to synthesize QDs when exposed to these oxidizing heavy metals. A time dependent change in fluorescence emission color, from green to red, was detected on cells exposed to metals. Biosynthesis was observed in cells incubated to different temperatures and high metal concentrations. Electron microscopy of treated cells revealed nanometric electron-dense elements and structures resembling membrane vesicles mostly associated to periplasmic space, particularly at cell poles. Purified biosynthesized QDs displayed broad absorption and emission spectra, characteristic of biogenic Cd nanoparticles. Our work presents a novel and simple biological approach to produce green QDs at room temperature by using metal resistant Antarctic bacteria, highlighting the unique properties of these microorganisms as potent natural producers of nano-scale materials and promising candidates for bioremediation purposes.

PP01.15

Radio-Resistant Breast Cancer Cells Exhibit Increased Population of Cancer Stem Cells and Highly Invasive Properties

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Abstract: Cancer patients are treated using combination therapy, which consists of surgery, chemotherapy and radiotherapy. However, each therapy has inherent limitations that lead to therapeutic resistance and cancer recurrence, ultimately resulting in therapeutic failure. Recent studies suggested that cancer stem cells (CSCs) exist in solid tumors and contribute to therapeutic resistance and disease recurrence. Therefore, we aimed to investigate whether the proportion of CSCs is increased among radio-resistant breast cancer cells and whether these cells contribute to tumor invasiveness and therapeutic resistance. MDA-MB-231 cells were irradiated 25 times (2 Gy each; 50 Gy total) to generate radio-resistant MDA-MB-231 (RT-R-MDA-MB-231) cells. Then, we confirmed the radio-resistance of RT-R-MDA-MB-231 cells by measuring cell viability after exposure to fractionated irradiation (2, 4, 6, or 8 Gy). As expected, RT-R-MDA-MB-231 showed the resistance to radiation and increased the levels of CSC markers Notch, Oct3/4 and aldehyde dehydrogenase-1. RT-R-MDA-MB-231 increased ICAM-1 and VCAM-1 levels, resulting in enhanced migration and adhesion to endothelial cells (ECs) compared to MDA-MB-231. Moreover, RT-R-MDA-MB-231 significantly increased invasiveness through ECs by inducing MMP-9, Snail-1 and b-catenin and by downregulating E-cadherin compared to MDA-MB-231. In addition, RT-R-MDA-MB-231 induced HIF-1a expression and LOX secretion, which are involved in the premetastatic niche formation, and showed resistance to chemotherapeutics such as tamoxifen. Finally, mice harboring RT-R-MDA-MB-231 xenografts showed enhanced tumor growth and higher levels of CSC markers compared to those harboring MDA-MB-231 xenografts. These results suggest that radio-resistant breast cancer cells exhibit enhanced invasiveness and resistance to cancer therapy due to the presence of CSCs.

PP01.16

A Bacterial Strategy to Stay in Glucose-Rich Niches

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Abstract: Flagellar motility is one of potential virulence factors in many pathogenic bacteria. Although the presence of preferred sugars such as glucose has been known to prevent flagellar motility in some bacteria, the underlying molecular mechanism remains unknown. Here we uncover the mechanism of glucose-mediated inhibition of flagellar motility in *Vibrio vulnificus*, a human pathogen causing septicemia. In the presence of glucose, enzyme IIA^{Glc} ($EIIA^{Glc}$) of the phosphoenolpyruvate:sugar phosphotransferase system inhibits the polar localization of FapA (flagellar assembly protein A) through a direct interaction. A loss or delocalization of FapA resulted in a complete failure of flagellar synthesis and motility. However, when glucose is depleted, $EIIA^{Glc}$ is phosphorylated and releases FapA. Free FapA is then localized back to the pole, where it activates the flagellar assembly. Together, these data provide new insight into a bacterial strategy to reach and stay in a glucose-rich environment.

PP01.17

Protective Effect of Vitamin D3 against Prednisolone-Induced Neurotoxicity in Rats

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Abstract: Neurotoxicity has recently been recognized as a complication of long-term glucocorticoid (GC) administration. However, the precise molecular mechanisms underlying GC-induced brain injury remain unclear. More evidence is needed for elucidating neuroprotective role of vitamin D_3. The aim of our study was to model glucocorticoid-induced brain injury and evaluate vitamin D_3 efficacy in correcting prednisolone-induced neurotoxicity. Female Wistar rats received prednisolone (5 mg/kg of b.w.) with and without 100 IU of D_3 (for 30 days). The levels of vitamin D_3 receptor (VDR), GC receptor (GR), pNF-κB p65, 1α-hydroxylase (CYP27B1), caspase-3 and nitrated proteins in brain tissue were determined by western blot analysis. The levels of VDR and NF-κB RNAs were measured by quantitative RT-PCR. $25OHD_3$ content in the serum was assayed by ELISA. Prednisolone induced an elevation of tyrosine nitration in the brain, reflecting NO-mediated neuronal toxicity that was accompanied by a significant increase in the level and transcriptional activation of NF-κB. It was shown a marked increase in caspase-3 level, indicative of cell apoptosis. An enhancement of VDR expression was observed concurrently with a marked increase in the level of GR, accompanied by a rise in CYP27B1 expression. All changes were associated with a 66% decrease in serum content of $25OHD_3$ that points to vitamin D_3 deficiency. Vitamin D_3 co-administration prevented abnormalities in the brain, indicating that D_3 deficiency can mediate prednisolone-evoked impairment in the brain. Thus, prednisolone-induced neurotoxicity is associated with the impairment of vitamin D_3 endocrine system and can be ameliorated by vitamin D_3 treatment.

PP01.18

Antidiabetic Potential and Proteomic Analysis of Parkia Biglobosa Protein Isolate in Streptozotocin-Induced Diabetic Rats

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Abstract: This study was designed to investigate the possible protective role of *Parkia biglobosa* protein isolate (PBPi) against streptozotocin-induced diabetic rats. PBPi (200 or 400 mg/kg body weight) was given orally by gavage or insulin (5 U/kg, i.p.) was administered daily to STZ-induced diabetic rats for 28 days. The degree of protection was evaluated using biochemical parameters such as fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL-c), low-density lipoprotein (LDL-c), thiobarbituric acid reactive substances (TBARS), serum total protein, serum transaminases (ALT and AST), total glutathione (total GSH), glutathione-S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) activities as well as serum interleukin-6 (IL-6). Total proteins extracted from rat liver were separated on one-dimensional polyacrylamide gel electrophoresis. The protein bands in the region of 10 – 15 kDa were altered by the different treatments; these bands were selected and cut for in-gel digestion and peptide extraction. HPLC fingerprint of PBPi revealed eleven distinct peaks. Oral administration of PBPi at the tested doses significantly ameliorated STZ-induced elevated levels of FBG, TC, TG, serum IL-6, ALT, AST and hepatic TBARS levels as well as concomitant increase in HDL-c. Serum total protein as well as the hepatic antioxidants (Total GSH, GST, SOD, CAT) were markedly restored in a dose dependent manner. Database search with the Mascot algorithm positively identified four (4) differentially expressed proteins. Taken together, this study provides significant insights into some of the mechanisms underlying the efficacy of PBPi in the treatment and management of diabetes.

PP01.19

The Development of Novel Pro-Drugs to Target Signalling Pathways Involved in Tumour Cell Migration.

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Abstract: Metastasis of cancer cells and the formation of secondary tumours is extremely problematic in the clinic, accounting for approximately 90% of human cancer deaths. The aggressive phenotype of many cancer cells rapidly leads to drug

resistance and highlights the need for a more targeted approach to treatment. Previous research has revealed important roles for Mnk1 and mTOR signalling in the translation of proteins involved in cell migration. Using Western blotting and trans-well cell migration assays, the work described here shows initial characterisation of small molecule inhibitors developed using rational drug design based on structural modelling and docking analysis. Following optimisation, future work will involve the synthesis of novel hybrid molecules allowing us to deliver a multi-hit attack on signalling pathways regulating migration in tumour cells. Our work will increase the treatment options and understanding of the pathways and mechanisms involved in tumour cell proliferation and migration.

PP01.20

In Vitro Detoxification Assessment of Bioactive Isoflavones in Rat and Human Liver Microsomes for Evaluating Safety

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Abstract: Formononetin (FMN) and Biochanin A (BCA) are the principal isoflavones present in commercially available extracts of red clover that are widely been consumed for various health benefits. We investigated the in vitro effects of FMN and BCA on catalytic activity of human/rat cytochrome P450 enzymes to assess the drug interaction potential of red clover. IC50 and Ki values of FMN and BCA for CYPs were determined in human/rat liver microsomes. FMN and BCA showed concentration-dependent inhibition of CYP1A2 activity with IC50 values of 13.42 and 24.98 ×M in human liver microsomes and 38.57 and 11.86 ×M in rat liver microsomes, respectively. The mode of inhibition of human CYP1A2 by FMN was found to be competitive with apparent Ki value of 10.1341.96 ×M. FMN also inhibited human CYP2D6. BCA exerted moderately inhibitory effects on human CYP2C9. The predicted in vivo inhibition for CYP1A2 was insignificant (R value <1.1) at hepatic level while at intestinal level, it was significant (R value >11). The inhibitory effects on other CYPs were found to be minimal. Red clover may be considered safe to be consumed along with co-prescribed medications; however, precaution must be taken while co-administering it with CYP1A2 substrates.

PP01.21

Do GRIK2 and TBP Genes Show Any Effect on the Age of Onset of Motor Symptoms in Patients with Huntington's Disease?

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Abstract: Huntington's disease (HD) is a rare progressive and fatal genetic neurodegenerative disease caused by expanded CAG repeats in the *HTT* gene. The CAG expansion accounts for about 70% of the variation of the age of onset of the disease. Modifier genes and/or environmental factors account for the rest of the variation in age of onset. We aimed to investigate the correlation between the modifier genes *GRIK2* and *TBP* and the age of onset of motor symptoms in Brazilian HD patients. 104 individuals were tested for HD: 72 tested positive for HD. In expanded alleles, CAG repeats ranged from 39 to 62 (mean 4544). This is a descriptive transversal study of HD in Brazil. The PCRs were performed using specific primers to analyze the target genes *TBP* (CAG/CAA repeats) and *GRIK2* (TAA repeats) and the amplicons were determined by fragment analysis by capillary electrophoresis. There was no significant correlation between age of onset of the motor symptoms and the size of the *TBP* polymorphic region (p= 0.32). As well as, there was no significant correlation between age of onset of the motor symptoms and the size of the *GRIK2* polymorphic region (p= 0.26). Only the number of CAG repeats in *HTT* had a strong correlation with the age of onset of HD in this group of patients (r =-0.81 and p<0.001). The next step of this study is to investigate the HD DNA haplotypes in these patients using a panel of 96 SNPs located across the HD gene region.

PP01.22

Mechanism Analysis of Effect of Proteoglycan on Cell Viability in Human Dermal Fibroblast

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Abstract: Proteoglycan, which is one of the glycoproteins, localizes to the cell surface and the extracellular matrix and has various functions. In recent studies, it has been gradually revealed that proteoglycan interacts with various cell growth factors and morphogens and regulates cellular functions. Previously, we have reported that salmon nasal cartilage proteoglycan increases cell viability in normal human dermal fibroblast (NHDF). However, the mechanisms that control cell viability are not yet understood. In the present study, to investigate the mechanism leading to increase of cell viability in NHDF by salmon nasal cartilage proteoglycan and elucidate possible physiological roles of the proteoglycan, the following experiments were conducted. Exp.1: To confirm the action of proteoglycan in NHDF, we investigated the dose and time-dependent effect of proteoglycan on the viability of NHDF. Proteoglycan was found to dose and time-dependently increase the viability of NHDF. Exp.2: To investigate whether the effect of proteoglycan on the cell viability is via core protein of proteoglycan, we analyzed effect of boiled proteoglycan on the viability of NHDF. The effect of proteoglycan on the cell viability was found to be heat stable, suggesting that the effect is not via core protein of proteoglycan. Exp.3: To investigate the mechanism of action of proteoglycan on cell viability, we analyzed cell growth signaling transduction. Proteoglycan strongly stimulated activation of cell growth signaling. The overall findings suggest that the glycosaminoglycan of salmon nasal cartilage proteoglycan plays a role in maintaining cutaneous function by up-regulating cell growth signaling.

PP01.23

HnRNP L Promotes Cell Apoptosis by Enhancing the Translation of p53 Under DNA Damage

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Abstract: The tumor suppressor p53 is an essential gene to induce cell cycle arrest, DNA repair and apoptosis. Under normal cell condition, p53 remains very low level by Mdm2-mediated ubiquitination. However, in various cellular stress conditions, an accumulation of p53 is observed, which blocks cell cycle progression and induces DNA repair. Although many studies reported that post-translational regulation is main mechanism of p53 accumulation, it also has been reported that post-transcriptional regulation of p53 mRNA contributes to increase in p53 protein level. In this study, we demonstrate that hnRNP L plays a crucial role in p53 translation. We found that hnRNP L increases and binds to 5'UTR of the p53 mRNA in response to DNA damage. Increased hnRNP L caused enhancement of p53 mRNA translation. However, knockdown of hnRNP L inhibited the p53 protein synthesis and accumulation under normal or DNA damaged condition. In the hnRNP L knockdown condition, decrease in p53 level made cell be resistant to apoptosis and arrest at G2/M phase under DNA damage. Therefore, our findings suggest that hnRNP L functions as a positive regulator of p53 translation and promotes cell cycle arrest and apoptosis.

PP01.24

Hydroquinone-Induced FOXP3-ADAM17-Lyn-Akt-p21 Signaling Axis Promotes Malignant Progression of Human Leukemia U937 Cells

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Abstract: Hydroquinone (1,4-benzenediol; HQ), a major marrow metabolite of the leukemogen benzene, has been proven to evoke benzene-related hematological disorders and myelotoxicity *in vitro* and *in vivo*. The goal of the present study was to explore the role of FOXP3 in HQ-induced malignant progression of U937 human leukemia cells. U937/HQ cells were prepared by continuous exposure of parent U937 cells to HQ. Proliferation and colony formation of U937/HQ cells were notably higher than those of U937 cells. Ten-eleven translocation methylcytosine dioxygenase-mediated demethylation of the Treg-specific demethylated region in FOXP3 gene resulted in higher FOXP3 expression in U937/HQ cells than in U937 cells. FOXP3-induced miR-183 expression reduced β-TrCP mRNA stability and suppressed β-TrCP-mediated Sp1 degradation, leading to up-regulation of Sp1 expression in U937/HQ cells. Sp1 up-regulation further increased ADAM17 and Lyn expression, and ADAM17 up-regulation stimulated Lyn activation in U937/HQ cells. Moreover, U937/HQ cells showed higher Lyn-mediated Akt activation and cytoplasmic p21 expression than U937 cells did. Abolishment of Akt activation decreased cytoplasmic p21 expression in U937/HQ cells. Suppression of FOXP3, ADAM17, and Lyn expression, as well as Akt inactivation, repressed proliferation and clonogenicity of U937/HQ cells. Together with the finding that cytoplasmic p21 shows anti-apoptotic and oncogenic activities in cancer cells, the present data suggest a role of FOXP3/ADAM17/Lyn/Akt/p21 signaling axis in HQ-induced hematological disorders.

PP01.25

Higher VEGF in Malignant Pleural Effusion Predicting Distant Metastasis Implies Benefit from Therapy Targeting VEGF Pathway

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Abstract: Background: Rapidly growing cancer cells secrete growth-promoting polypeptides and have increased proteolytic activity, contributing to tumor progression and metastasis. Their presentation in malignant pleural effusion (MPE) and their predictive value for the outcome of pleurodesis and survival were studied. Methods: Between February 2011 and March 2012, MPE samples were prospectively collected from 61 patients. Twenty-five patients with non-malignant pleural effusion in the same period were included as controls. Pleural fluid osteopontin (OPN), vascular endothelial growth factor (VEGF), and urokinase-type plasminogen activator (uPA) concentrations were measured. Results: Patients with MPE had higher pleural fluid OPN, VEGF, and uPA concentrations than those with non-malignant pleural effusion, but only differences in VEGF were statistically significant (p=0.045). Patients with distant metastases had significantly elevated pleural fluid VEGF concentrations than those without (p=0.004). Pleural fluid OPN, VEGF, and uPA concentrations were positively correlated in most patients. However, there was no significant difference in pleural fluid OPN, VEGF, and uPA concentrations between patients with successful pleurodesis and those without. There was also no significant difference in cancer-specific survival between sub-groups with higher and lower pleural fluid OPN, VEGF, or uPA concentrations. Patients with successful pleurodesis had significantly longer cancer-specific survival than those without (p=0.015). Conclusions: Pleural fluid OPN, VEGF, and uPA concentrations are elevated in MPE but are not satisfactory predictors of pleurodesis outcome or survival. Patients with higher pleural fluid VEGF concentration have higher risk of distant metastasis. Evaluating the benefits of therapy targeting the VEGF pathway in these patients warrants further studies.

PP01.26

Interaction of Aspergillus Fumigatus Conidia with Human Airway Epithelial Cells

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Abstract: Background: *Aspergillus fumigatus* is an opportunistic fungal pathogen that can cause life-threatening pulmonary disease in immunocompromised patients. Interaction of *A. fumigatus* conidia with airway epithelial cells (AECs) may contribute to pathogenesis. Our objective was to analyze dual organism transcriptomic data to yield candidate genes that may play a role in the interaction between *A. fumigatus* conidia and the human bronchial epithelial cell line, 16HBE14o-. **Methods**: The transcriptional responses of human and fungal cells upon co-incubation (6 hours) were obtained from dual organism cDNA microarray data. Two R packages, LIMMA and mixOmics, were used to generate lists of genes displaying significant differential expression and highly correlated gene pairs. Expression patterns of these genes were tested for replication in expression data collected from a related experiment in which human-fungal co-cultures (6 hours) were subjected to FACS to yield samples of human cells associated or unassociated with conidia. **Results**: Differential expression analysis identified 66 up-regulated and 108 down-regulated genes in humans, and 35 up-regulated and 81 down-regulated genes in *A. fumigatus* ($P<0.05$). Out of 81 gene pairs identified that were highly correlated ($R^2>0.95$), a human gene called MOP-1 (function unknown) was also differentially expressed in the FACS experiment. MOP-1 expression was negatively correlated with five *A. fumigatus* genes, including metabolic enzymes such as triose phosphate isomerase and glycerol dehydrogenase. **Conclusion**: We identified five *A. fumigatus* genes that were negatively correlated with MOP-1 expression in epithelial cells. Current studies are investigating interactive transcriptomes using primary human AECs co-cultured with conidia.

PP01.28

Antidiabetic and Hypolipidemic Effects of Methanolic Extract of Adiantum Lunulatum in Alloxan Induced Diabetic Rats

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Abstract: Diabetes associated dyslipidemia has been a life-threatening disability in worldwide. The present study was designed to investigate the effect of methanolic whole plant extract of *Adiantum lunulatum* (MWAL) on alloxan-induced diabetes with dyslipidemia in rat model and also antioxidant status. Acute toxicity study of MWAL was done for four days and oral glucose tolerance test was performed in normal male Swiss albino rats. Diabetes was induced by a single intraperitoneal (i.p) injection of alloxan (120 mg/kg). After induction of diabetes, the rats were divided into six groups each comprising a minimum of five rats. MWAL was treated at the doses of 250mg/kg & 500mg/kg b.w. One of the groups was treated with Metformin 850mg/70kg b.w. (standard) and combination of dose (Metformin 425mg/70kg and MWAL 250mg/kg) was also studied for two weeks. Blood glucose level and serum lipids profile were estimated after two weeks. We also evaluated the antioxidant potentials. All the tested doses have been found effective in reducing fasting blood glucose level, TC, TG, LDL and improving HDL significantly. Highest activity was shown by the treatment MWAL 500mg/kg b.w. which was statistically significant (PM0.05). Total phenolic content and total flavonoid content of the extract were 308.83 mg of gallic acid equivalent and 48.08 mg of quercetin equivalent per gm of dried extract. MWAL showed considerable total antioxidant activity and reducing capacity. In DPPH scavenging assay the MWAL showed 84.33% scavenging having IC_{50} of 79.27×g/ml. Data obtained in this study demonstrated the considerable amount of antihyperglycemic, hypolipidemic and antioxidant effects.

PP01.29

Connection of Hedgehog Signaling Pathway and Steroidogenesis in the Liver

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Abstract: The liver has multiple anabolic and catabolic functions. Novel results showed that Hedgehog signaling, commonly associated with embryogenesis, development and cancer, is active in hepatocytes and acts as a master regulator of zonation in the adult liver (Gebhardt & Matz-Soja, WJG, 2014). We are interested in how this signaling pathway is connected to sex-specific regulation of gene expression. We generated two transgenic mouse strains with an inactivated (SAC) and constantly-activated (Ptch1LC1) Hedgehog pathway in hepatocytes, respectively. We surprisingly found a regulation of steroidogenic genes in hepatocytes of mice with aberrant Hedgehog signaling; yet it was assumed that steroidogenesis occurs in the liver only during embryogenesis and is down-regulated afterwards. A down-regulated Hedgehog pathway results in the up-regulation of some of the steroidogenic genes and vice versa. Expression of Cyp17a1, a central regulator of steroidogenesis, is up-regulated in SAC and decreased in Ptch1LC1 knockouts. Furthermore, we observed a clear correlation between regulation of estrogen receptor (Esr1) and Cyp17a1 expression in both mouse models. A transcription factor binding site analysis revealed GLI binding sites in the promotor region of Esr1, but not for Cyp17a1. It needs to be further investigated how the regulation of steroidogenesis is influenced by Hedgehog signaling and how Cyp17a1 and Esr1 expression is regulated by each other. Collectively, the experiments showed a clear influence of the morphogenic Hedgehog pathway on the regulation of steroidogenesis in the liver. These unexpected findings are promising for future studies to improve our understanding of the gender dimorphism of regulatory mechanisms in liver.

PP01.30

Importin-α Variants Comparison by Crystallographic and Calorimetric Techniques

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Abstract: The communication between the nucleus and the cytoplasm occurs through transport mechanisms that allow the passage of molecules through pores present in the nuclear envelope. The best characterized nuclear import pathway requires positively charged sequence(s) within the cargo protein, known as classical nuclear localization (NLS) sequence which binds to the importin-α (Impα) protein. Structures of Impα from different organisms (e.g. yeast, rice, mouse and human) have been determined, revealing that this receptor posses a conserved structural scaffold. Amino acid sequence analysis demonstrated that is possible to classify them into three families: α1, α2, and α3. Some studies have also demonstrated that the Impα mechanism of action may vary significantly for different families. Thus, in this work we performed crystallographic and calorimetric comparison between two families of Impα to different NLS peptides in order to define specificities of the Impα from *Mus musculus* (MmImpα - type α2) and the Impα from *Neurospora crassa* filamentous fungus (NcImpα - type α1). The comparison of these data with previous studies on Impα proteins led us to demonstrate that NcImpα possess specific features that are distinct from MmImpα but exhibits important similarities to rice Impα (type α1), particularly at the minor NLS binding site. Financial support: FAPESP (proc. 2013/24705-3), CNPq and CAPES.

PP01.31

New Approach for Cell Imaging and Apoptosis Detection by Fluorescent Carbon Nanoparticles

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Abstract: In the nanotechnology field, much interest was focused on the new carbon nanomaterials for cell imaging. Recently discovered inorganic carbon nanoparticles (CDots) due to their excellent fluorescence characteristics and biocompatibility have ample opportunities for application in imaging and for detection of functional transformations in living cells. Their distinctive features, such as high brightness, small sizes, high biocompatibility and methods of their preparation present a good alternative to other nanoscale materials. The focus of our research was to determine the possibility of using CDots as the easily available probes for detection of apoptotic cells [1]. With these tools we demonstrate that both intact and apoptotic cells can be easily visualized by CDots. Using the different methods of sample preparation, they show the ability for labeling various structural compartments of the cell. For living cells there are the intracellular vesicles and lysosomes. In contrast, in fixed cells the nucleus is labeled preferentially. The fact that apoptotic cells accumulate strongly increased amount of CDots can be efficiently used in flow cytometry for characterizing the cell populations regarding the relative amount of apoptotic cells in different experimental conditions. The application of such cheap and easily accessible nanoparticles, in combination with previously showed possibility of using these fluorophores for super resolution method SOFI [2], provides more opportunities to simplify the popular methods of cell labeling and detection. [1] Dekaliuk, et al. *J. Nanobiotechnology*, vol. 13, no. 1, p. 86, 2015. [2] A. M. Chizhik, S. Stein, M. Dekaliuk,et al. *Nano Lett.*, pp. 8–13, 2015.

PP01.32

Voluntary Wheel Running Modifies Neuroinflammatory Status: The Role of Monocyte Chemoattractant Protein-1

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Abstract: Physical activity (PA) is crucial for maintaining a healthy body and brain. Moderate PA induces anti-inflammatory effects in the peripheral tissues, including decreased levels of circulating pro-inflammatory cytokines, and increased levels of anti-inflammatory cytokines. However, in contrast with the periphery, very little is known about the effects of PA on neuroinflammation. Here, we bridge this knowledge gap by testing the hypothesis that voluntary wheel running (VWR) has an effect on brain cytokine levels and markers of glial cell activation in female C57BL/6 mice. We investigated the role of monocyte chemoattractant protein (MCP)-1 in mediating the neuroimmune responses to VWR, by using MCP-1 knock-out mice. We demonstrated that, compared to their sedentary counterparts, mice engaged in seven weeks of VWR had increased brain levels of pro- and anti-inflammatory cytokines, increased markers of glial cell activation and enhanced toll-like receptor (TLR) 4 expression in the brain. MCP-1 knock-out mice from the VWR group displayed a different cytokine expression profile and demonstrated a reduction in the expression of glial cell activation markers, but showed no difference in TLR4 expression, compared to their wildtype counterparts. We propose that the changes in brain cytokine levels and glial cell activation represent enhanced immune response in VWR mice, as opposed to a suppressed immune system in the sedentary group, and that these responses to VWR are at least partially reliant on MCP-1. Therefore, MCP-1 may play a role in mediating the relationship between a physically active lifestyle and the reduced risk for developing neurodegenerative diseases.

PP01.33

Neuro-Protective Capacity of Dental Pulp Stem Cells in an in Vitro Model of Parkinson's Disease

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Abstract: Dental pulp stem cells (DPSC) have recently became sensational topic of discussion and extensive amount of works were carried out to comprehend them. Owing to their inherent propensity toward neuronal-lineage, huge emphasis was placed on DPSC so that they can be utilized for cell replacement therapy (CRT) especially towards neurological-related concerns. However, apart from being able to differentiate into cell-linage of interest, the transplanted cells must ideally able to withstand harsh conditions in that specific microenvironment. As such in this study, the neuroprotective capacity of DPSC was evaluated in an *in vitro* model of Parkinson's disease (PD). Using IMR-32 and EOC2 cell lines, the optimum MPTP concentration to simulate PD model was identified. They were then co-cultured with DPSC either with or without pre-conditioning treatment and further evaluated in terms of cell count, viability, DNA damage, production of reactive oxygen species (ROS) as well as expression of inflammatory cytokines. As expected, the viability and cell count of both cell lines displayed decrement in a dose-dependent manner. With introduction of DPSC, the neurotoxicity was significantly attenuated as compared to control especially in pre-conditioned DPSC (DPSC-p; $p<0.05$). In addition, inflammatory cytokines were also found to be differentially expressed upon introduction of DPSC and DPSC-p in the said model. As a whole, this study had demonstrated the neuroprotective capacity of DPSC and pre-conditioning treatment has significantly augmented their neuroprotective behaviour. These findings are expected to improve our current understanding in application of DPSC, particularly in those related to neurological issues.

PP01.34

Loop Closure and Kinase Selectivity in Lung Cancer

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Abstract: Lung cancer is a leading cause of cancer deaths worldwide. Somatic mutations in tyrosine kinase receptors that causes aberrant signalling have been implicated in the development of lung cancer. Two such receptors, EGFR and FGFR kinases are directly involved in many cases of aggressive metastasis and drug resistance. The FGFR kinase family consists of four highly conserved receptor proteins (FGFR1 – FGFR4). FGFR pathways are the main cause of resistance to chemotherapy in non-small cell lung cancer patients, and 22% of them show over-expression of FGFR1. There are a number of small molecules in phase III clinical trials that target not only FGFR but also other kinases. A wide range of EGFR mutations are linked to lung cancer development in never-smokers or former smokers. The two most common mutations are exon 19 deletions and the point mutation L858R in exon 21. Many patients harbouring L858R acquire a secondary T790M mutation after treatment with gefitinib/erlotinib resulting in drug resistance. In the past few years AstraZeneca have developed drugs that target specific proteins, eg; AZD4547 (FGFR1 selective) and AZD9291 (selectivity for T790M/L858R EGFR). In an effort to design our own novel and selective inhibitors, we solved the structures of AZD4547 and AZD9291 in complex with FGFR and EGFR respectively. In both cases, the phosphate binding loop (P-loop) of the proteins forms an unusual "bent" structure wrapped closely around these inhibitors. We speculate that the ability of these compounds to induce P-loop closure is an important part of their respective selectivity mechanisms.

PP01.35

Crystal Structure Reveals the Molecular Basis of STAM2-Binding Specificity of the HD-PTP Bro1 Domain Regulating EGFR Sorting

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Abstract: EGFR is a receptor tyrosine kinase that plays a key role in cell survival and proliferation signaling. For regulating its activity, EGFR is endocytosed and recycled back to the plasma membrane or sorted into multivesicular bodies and finally into lysosomes to be degraded. The endosomal membrane-localized ESCRT machinery do a pivotal role in EGFR trafficking. HD-PTP is a non-receptor type protein tyrosine phosphatase and known to be critically implicated in EGFR trafficking. HD-PTP contains a Bro1 domain that binds the ESCRT components STAM2 and CHMP4B. Interestingly, Brox and Alix, two other Bro1 domain-containing human proteins, interact with CHMP4B but not with STAM2. Herein, we determined the crystal structure of the HD-PTP Bro1 domain in a complex with the STAM2 core region. The HD-PTP-binding STAM2 fragment forms an amphipathic helix and is accommodated in the hydrophobic pocket of the Bro1 domain. Despite that CHMP4B binds to the concave pocket of Bro1 domains in a similar manner, we found a key structural differences that modulates STAM2 and CHMP4B binding specificity of the Bro domains; Thr145 of HD-PTP, corresponding to Arg145 of Brox and Lys151 of Alix, is revealed to be a determinant residue enabling HD-PTP to bind STAM2. This is because only Thr145 of HD-PTP does not cause steric hindrance with the STAM2 helix. Indeed, the Brox- or Alix-mimicking mutations lead to the blockade of the intermolecular interaction between HD-PTP and STAM2. Therefore this study provides the rational explanation of how HD-PTP specifically recognizes the ESCRT machinery to control EGFR trafficking.

PP01.36

Different Anti-Apoptotic Effects of Allergen on Eosinophil Apoptosis between Atopic and Non-Atopic Asthmatic Subjects

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Abstract: House dust mite is a major allergen in allergic diseases such as asthma. In this study, we investigated the effects of house dust mite on constitutive eosinophil apoptosis in normal and asthmatic subjects. We classified asthmatic subjects into those with atopic and non-atopic asthma depending on the presence of DP-specific IgE or/and DF-specific IgE in serum. Both blood and bronchoalveolar lavage fluid (BALF) eosinophils in atopic asthma were elevated when compared with normal and non-atopic eosinophils. *Dermatophagoides pteronissinus* extract (DP) inhibited constitutive eosinophil apoptosis of atopic asthmatic subjects, but not that of normal and non-atopic subjects. DF had no effect on eosinophil apoptosis in normal and asthmatic subjects. Anti-apoptotic signaling mediated by DP in atopic asthma was associated with the TLR4/PI3K/Akt/ERK/NF-κB pathway. Activation of procaspase 3 and procaspase 9 was delayed by DP stimulation. Our results indicate that DP induces eosinophilic inflammation by inhibiting eosinophil apoptosis, and that the inhibitory effect is associated with exposure of asthma subjects to DP. A better understanding of the difference between atopic and non-atopic asthma will help elucidate the pathogenesis and develop methods for treatment of asthma.

PP01.37

KPNB1-Mediated Nuclear Import Is Required for Inflammatory Cytokine Expression, Invasion and Survival of Cancer Cells

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Abstract: Karyopherin β1 (KPNB1) is a nuclear import protein involved in the transport of transcription factors and other proteins containing a nuclear localisation sequence. Elevated KPNB1 expression has been reported in cancer and transformed cells. Transcription factors such as NFκB and AP-1 contain a NLS and have been suggested to require KPNB1 for nuclear import. These transcription factors initiate the expression of multiple cytokines and factors associated with inflammation and cancer cell biology. An inflammatory microenvironment, a hallmark of cancer, contributes to factors such as sustained proliferation, invasion and neoangiogenesis. Our study aimed to investigate the effect of inhibiting nuclear import via KPNB1 as a potential anti-cancer and anti-inflammatory approach using siRNA and the novel small molecule, inhibitor of nuclear import- 43 (INI-43). We found that inhibiting KPNB1 lead to reduced migration and invasion of cervical cancer cells while extended inhibition caused decreased proliferation and apoptosis. KPNB1 is essential for the translocation of NFκB into the nucleus as inhibition of nuclear import resulted in its cytoplasmic retention and decreased transcriptional activity of both NFkB and AP-1. DNA-binding studies confirmed a reduced binding-ability or presence of NFkB in the nuclear extract of KPNB1-inhibited cells. Consequently reduced IL-6, IL-1β, TNF-α and cJun target gene expression was observed. INI-43 was able to inhibit tumour growth in an ectopic xenograft mouse model while the effect on inflammation and cancer cell biology was further explored using immunohistochemistry. Our study provides evidence that inhibiting KPNB1 has anti-inflammatory and anti-cancer effects and shows promise as a chemotherapeutic target.

PP01.38

Involvement of MAPK Network Signaling and Feedback Mechanisms in Prion Formation

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Abstract: Prion diseases are fatal neurodegenerative disorders characterized by the accumulation of a misfolded and aggregated form of the host encoded cellular prion protein ($PrP^{C)}$ in the central nervous system of human and a wide variety of animals. Beside sporadic and genetic, prion diseases may also be infective. Indeed, PrP^{C} abnormal isoform, namely PrP^{Sc} or prion, may be transmitted via the consumption of prion-infected food or via iatrogenic means. Given the infective nature of prions, the identification of the pathogenic mechanisms and the conversion factors involved in PrP^{Sc} formation is of paramount interest. The aim of this project is to identify the intracellular signaling pathways involved in prion spread and propagation. Previous studies highlighted an altered ERK1/2 activation and intracellular localization in mouse hypothalamic GT1-1 cells infected with scrapie prions (ScGT1-1) (Didonna *et al.*). Activation of the MEK/ERK signaling pathway was shown to promote PrP^{Sc} formation in ScGT1-1 cells, while its inhibition led to clearance of prions in the cells but had no affect on PrP^{C} levels (Allard *et al.*). Furthermore, opposing effects of activation of the ERK and *c-jun*/JNK pathways on prion formation was found. As first objective, we investigated whether calcium- or endocytosis-mediated mechanisms are responsible for prion-induced ERK activation in ScGT1-1 cells. Different calcium channels blockers and endocytosis inhibitors were used and pERK, p*c-jun* and PrP^{Sc} levels measured. Secondly, western blotting analyses were performed to study ERK downstream targets, e.g. CREB and Elk1. Finally, the expression level of target transcripts was evaluated by RT-qPCR analysis.

PP01.39

Engineering Platelets for the Delivery of RNA

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Abstract: Local delivery of therapeutics to sites of vascular damage remains a major challenge in managing hemorrhage and cardiovascular disease. Nature has in part overcome this challenge by using platelets as delivery vehicles for small molecules and biological macromolecules that regulate coagulation and inflammation. This includes the release and transfer of RNA-containing microparticles, leading to altered protein expression in nearby cells. The natural role of platelets as delivery vehicles provides strong motivation for utilizing these cells as carriers of nucleic acid-based therapeutics. Previous work has shown that platelets can be transfected with synthetic miRNA, however direct modification of platelets with mRNA has not yet been achieved. My project aims to develop ways to directly introduce mRNA to platelets, using lipid nanoparticles (LNPs) to deliver *in vitro* transcribed mRNA to platelets *ex vivo*. Using this approach, I have shown that exogenous mRNA encapsulated within LNPs was internalized by platelets, with minimal platelet activation and aggregation. Future work will focus on testing whether this mRNA can be translated directly by the platelets, or be transferred to and utilized by other cell types. Not only will these platelets have potential uses as delivery vehicles for RNA-based therapeutics, but this system may present a novel experimental approach for directly modifying and studying platelet biology.

PP01.40

Alterations in Peripheral Blood Mononuclear Cells Phospholipid Content in Breast Cancer

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Abstract: Recent studies suggest a potential impact of changes in different subpopulation of immune cells on solid tumors and hematological malignancies resulted in the alteration of balance between pro-and anti-tumor immunity. It was hypothesized by us earlier that in such intricate system diseases, as human tumors, alterations in the cell plasma membrane (PM) lipid homeostasis in the peripheral blood crude mononuclear cells (MNC) may possibly represent some information useful for detection and evaluation of diverse cancers as well as for the discovery of new modes of chemotherapy treatment.The aim of this study was to investigate the quantitative changes in the phospholipid (PL) content of MNC PM fraction in breast cancer (BC) compared to norm.Data obtained indicate that eight different PL fractions, identified by TLC method in the PM of blood MNC, were reliably altered in all cancer patients compared to healthy individuals. Particularly, it was shown significant increase in the content of lysophosphatidylcholine fraction in BC compared to norm. Importantly, regular and distinctly individual for each patient disturbance in the contents of different PL fractions reveled in BC were identical with those observed earlier in leukemia and some other forms of solid tumor.We conclude that pathological alterations in PLs content of crude MNC PMs have been similarly involved in the onset and evolution of diverse forms of cancer and can be used for early detection and definition of cancer as well as for personalized correction of disease treatment modes.

PP01.41

Biochemical and Histological Changes of Postpartum Rats Exposed to Natron

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Abstract: The prevalence of Peripartum cardiomyopathy (PPCM) among northwest Nigerian women is put at 1 % probably due to customary puerperal practices, including consumption of a dry lake salt, natron. This study was designed to examine the effect of natron (kanwa) in the pathogenesis of PPCM. A total of 30 post-partum rats were randomly divided into five groups (I-V) of 6 rats each. Group I served as control, Group II-V were treated with 50mg/kg, 100mg/kg, 200mg/kg and 300mg/kg of Natron for a period of four (4) weeks. At the end of the experimental period, the rats were anesthetized and blood and tissue samples were collected for biochemical and histological assessment of cardiac, liver and kidney functions. The findings of the work revealed that treatment of rats with natron at dose of 100mg/kg, 200mg/kg and 300mg/kg caused significant increase ($p<0.05$) in cardiac function parameters with no significant ($p>0.05$) changes in the renal function parameters and significant increase ($p<0.05$) in some of the liver function parameters. Histological examinations revealed no effect on the kidney in all the treated groups, mild to moderate portal triaditis, a nonspecific signs which may not be related to the administered substance, in the liver of almost all the groups and myocyte hypertrophy in animals treated with 100mg/kg and above. The study suggests that consumption of natron for 4 weeks caused alteration in cardiac function parameters and thus may contribute to the pathogenesis of PPCM.

PP01.42

Towards Synthesizable High Affinity Human Pancreatic Alpha-Amylase Inhibitors - A New Class of Potential Anti-Diabetics

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Abstract: Without treatment, high blood glucose levels in diabetic patients lead to increased risk of cardiovascular disease, kidney failure, and nerve damage. Oral therapy to control hyperglycemia can potentially serve to delay the onset of diabetes in pre-diabetic patients, thereby increasing quality of life and reducing health care costs. However, current oral anti-diabetic drugs that control blood glucose levels show poor patient compliance, due to the non-specific inhibition of a wide range of glucosidases within the gut, which leads to significant side effects. Recent studies have shown that specific inhibition of human pancreatic alpha-amylase (HPA) with Montbretin A (MbA, Ki = 8 nM) has the potential to prevent starch degradation, therefore reducing such side effects. However, given that MbA is a complex flavonol glycoside, it is not well suited for pharmaceutical synthesis. We have determined the high resolution structure of HPA in complex with MbA and a deglycosylated form of MbA, designated as mini-MbA (Ki = 93.3 nM), which represents the inhibitor's core structure. Our current work is directed towards utilizing the novel nature of MbA inhibition to design related inhibitors that are more amenable to chemical synthesis. Furthermore, in tandem with related kinetic and structural analyses, these new inhibitor designs will be optimized with the goals of enhanced binding affinity and thereby ultimately defining a potential therapeutic that maximizes efficacy with a reduced side effects profile.

PP01.43

Human MSC Therapy Restores Cognitive Function and Improves Hippocampal Morphology in a Rat Model of Senile Neurodegeneration

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Abstract: Brain aging is associated with a progressive increase in the incidence of neurodegenerative diseases and deterioration of spatial learning and memory in aging rats (AR) and human. This makes this rodent species a suitable model to evaluate therapeutic strategies of potential value for correcting age-related cognitive deficits. Adult bone marrow-derived MSCs (BM-MSCs) have been reported as potential candidates for treatment of neurodegenerative disorders. Here, we investigated the therapeutic potential of MSCs to treat cognitive impairment in AR. Dil-labeled human BM-MSCs were intracerebroventricularly bilaterally injected to 27-month-old female rats. Experimental subjects were divided in 3 groups: Young-intact, Senile-intact and Senile-MSC. Using the Barnes maze we assessed hippocampus-dependent learning and spatial memory before and after cell injection. Also, we assessed recognition memory with the Novel Object Recognition test. Additionally, we performed time-course studies for MSCs integration and viability in the brain and assessed a set of hippocampal cell markers. MSC therapy increased goal hole exploration activity in senile rats as compared with intact counterparts. Immature neuron number in the hippocampal dentate gyrus was higher in treated animals. Time course studies (24 days) revealed that MSCs integrated into ependymal cell layer and even in brain parenchyma. The results suggest that MSC therapy partially reverse the decline in cognitive performance that occurs in AR and improves a number of morphological parameters in the hippocampus. We conclude that adult stem cells are a suitable biological tool for the treatment of age-related neurodegeneration.

PP01.44

Biochemical and Structural Analysis of SbnI, a Heme-Sensing Regulator of Iron Source Preference in Staphylococcus Aureus

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Abstract: *Staphylococcus aureus* is a prominent cause of infectious disease in humans. This can at least in part be attributed to the multitude of mechanisms by which it can acquire host iron, allowing *S. aureus* to infect a variety of tissues. Iron uptake is accomplished namely through heme uptake by the Isd (iron-responsive surface determinant) system and through the secretion of iron scavenging siderophores. Staphyloferrin B (SB) is a siderophore produced by *S. aureus* using the 9-gene *sbn* gene cluster for biosynthesis and efflux. The ninth gene product, SbnI, has recently been described as a heme-sensing regulator of SB production. SbnI can bind heme, which obviates interaction with DNA and restricts the production of SB and growth in iron restricted conditions. We present crystal structures of SbnI containing a short C-terminal truncation and of the *Staphylococcus pseudintermedius* SbnI homolog. These structures have revealed that SbnI forms a dimer through C-terminal domain swapping and can form a dimer of dimers under non-reducing conditions. Additionally, we show a link between heme and siderophore iron uptake as a heme-degrading protein of the Isd system, IsdI, can serve as a heme source for SbnI. These findings suggest that SbnI may serve as a major regulator of iron source preference in *S. aureus* and provide rationale for how *S. aureus* demonstrates heme iron preference. The data supports the model of SbnI as a heme dependent regulator of SB biosynthesis and offers a mechanism for how *S. aureus* can adapt to different iron-restricted niches within the host.

PP01.45

Role of MK5 in Cardiac Fibroblast Proliferation, Migration, and Expression of Inflammatory Cytokines and Chemokines

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Abstract: Cardiac fibroblasts are the most predominant cell type in the heart. They play a critical role in post-infarction cardiac repair following myocyte loss and are implicated in the pathogenesis of cardiac remodelling. MAP kinase-activated protein kinase-5 (MK5) is highly expressed in the heart but its physiological role is just beginning to be understood. MK5 haploinsufficient ($MK5^{+/-}$) mice showed reduction in pressure overload-induced collagen expression. Furthermore, scar rupture was more frequent in $MK5^{+/-}$ mice following myocardial ischemia induced by ligation of the left anterior descending coronary artery. Thus, MK5 may play a role in extracellular matrix remodelling. This study sought to probe the role of MK5 in cardiac fibroblast proliferative and migration. Cardiac fibroblasts were isolated from male $MK5^{+/+}$, $MK5^{+/-}$ and $MK5^{-/-}$ mice. Subconfluent cultures from passage 2 and 3 were used. Cell migration, assessed by scratch-wound assay, in response to serum and/or angiotensin-II was severely impaired in $MK5^{-/-}$ fibroblasts compared to $MK5^{+/+}$ and $MK5^{+/-}$. Cell migration was also reduced upon acute knockdown of MK5 with siRNA. In contrast, $MK5^{-/-}$ fibroblasts showed increased rates of proliferation. Interestingly, pathway-targeted qPCR microarrays revealed a decrease in the abundance of cytokine and chemokine mRNAs (e.g., IL1a, IL1b, IL1 receptor antagonist-2, IL4, IL10, CCL11, CXCR4, IFNγ, TNFa) in $MK5^{-/-}$ fibroblasts compare to $MK5^{+/+}$ fibroblasts, suggesting a possible role of MK5 in the inflammatory phase of cardiac repair. Hence, MK5 may be involved in cardiac fibroblast proliferation, migration and, possibly, myocardial remodelling.

PP01.46

Mitochondrial ROS Activate PI3K-AKT-mTOR Pathway and Induce mTOR-Mediated Autophagy during Muscle Differentiation

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Abstract: Skeletal muscle differentiation is a crucial process controlling muscle development and homeostasis. Mitochondrial reactive oxygen species (mtROS) rapidly increase during the muscle differentiation, which are recently magnified as critical intermediates of cellular signaling pathways, with which it has not yet been elucidated how it controls myogenic signaling. Autophagy, a lysosome-mediated degradation pathway, is recognized as important for intracellular remodeling of organelles and proteins during muscle differentiation. Here, we have demonstrated that the mtROS are required to myogenic autophagy, depending on PI3K-AKT-mTOR cascade. Activation of mTOR (S2448) induced autophagic signaling via mtROS-induced PTEN oxidative inactivation to render muscle differentiation, whereas MitoQ or rapamycin treatment impaired the myogenic processes. Conclusively, we suggest a novel regulatory paradigm that mTOR physiologically leads to autophagic reconstruction of cellular organization through mtROS-mediated signaling activation in muscle differentiation. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MEST) (No. 20110030072 and NRF2013R1A1A2061214).

PP01.47

Cysteine Cathepsins B and X Are Involved in Epithelial-Mesenchymal Transition

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Abstract: Lysosomal cysteine cathepsins B and X are unique among cysteine cathepsins due to their carboxypeptidase activity. Additionally, because of the extra structural element termed the occluding loop, cathepsin B can also act as endopeptidase. Dysregulation of expression and activity of both cathepsins B and X is associated with a variety of pathological processes including cancer. In cancer, they have an important role in number of processes including tumor invasion and metastasis. However, their role in epithelial-mesenchymal transition (EMT), process that has been recognized as an important step in progression of tumors, their invasion and metastasis, has not yet been explained. In the present study we evaluated involvement of cysteine cathepsins B and X in EMT on different cell lines. We observed the correlation between expression of EMT markers and cathepsins B and X as cell lines with higher levels of cathepsins expressed mesenchymal markers in contrast to those with their lower levels that expressed epithelial markers. Moreover, silencing and up-regulation of cathepsin B and X effected the expression of EMT markers. We showed that after treatment with transforming growth factor-β1 (TGF-β1) the induction of EMT was associated with increased expression of cathepsin B while the increase in cathepsin X was less pronounced. To conclude, cathepsins B and X, when expressed in higher levels, promote EMT, however their compensation role in TGF-β1 induced EMT was not evident. Furthermore, our results indicate that among two cathepsins cathepsin B has more important role in EMT and consequently invasion and migration of tumor cells.

PP01.48

Imaging Mass Spectrometry for the Analysis of 3D Tissue-Engineered Psoriatic Skin Models

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Abstract: Tissue-engineering has enabled the development of skin models. These living skin equivalents [LSEs], derived from primary human skin cells, self-assemble to form stratified layers comparable to human skin. LSEs offer treatment for burns patients following serious injury, avoiding the need for meshed skin grafts and donor skin. Additionally LSEs are used for toxicity screening with the ability to replace animal models for cosmetic and drug development. While there are still limitations to LSEs they provide a model for direct quantification of drug treatment and efficacy towards skin. MALDI-mass spectrometry imaging affords proteomic and lipidomic analysis associated within psoriatic and normal skin constructs. We have also used MALDI-MSI to image the effectiveness of drug treatments towards tissue-engineered psoriatic skin constructs. We have previously applied MALDI-MSI to the study of both ex-vivo human skin and 3D skin models. Much progress is being made towards the validation of lipids and other small molecules with the use of MALDI-MSI. MALDI-MSI affords a closer look, and increased understanding, for drug metabolism and distribution. Following treatment with pro-inflammatory cytokine IL-22 epidermal differentiation within the LSEs successfully modelled psoriasis *in vitro*. Additionally it was possible to observe the effect of therapy drug treatments towards both psoriatic skin and normal skin. The depth of penetration was observed. It was also possible to observe the psoriatic character of the LSEs through histology and immunohistochemistry profiling. The use of high mass accuracy and ion mobility separation capabilities enabled separation of isobaric species and precise profiling of the skin constructs.

PP01.49

Exploring Protein Stability and Aggregation by nanoDSF

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Abstract: We will introduce the Prometheus NT.48 instrument and its novel nanoDSF technology, which allows for parallel high-precision characterization of stability and aggregation parameters of biologicals. nanoDSF is an advanced Differential Scanning Fluorimetry technology. It detects smallest changes in the fluorescence of tryptophan present in virtually all proteins. The fluorescence of tryptophans in a protein is strongly dependent on its close surroundings. By following changes in fluorescence, chemical and thermal stability can be assessed in a truly label-free fashion. The dual-UV technology by NanoTemper allows for rapid fluorescence detection, providing an unmatched scanning speed and data point density. This yields an ultra-high resolution unfolding curves which allow for detection of even minute unfolding signals. Furthermore, since no secondary reporter fluorophores are required as in conventional DSF, protein solutions can be analyzed independent of buffer compositions, and over a concentration range of 200 mg/ml down to 5 ×g/ml. Therefore, nanoDSF is the method of choice for easy, rapid and accurate analysis of protein folding and stability, with applications in membrane protein research, protein engineering, formulation development and quality control.

PP01.50

Biomolecular Interaction Determination and Quantification by MicroScale Thermophoresis

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Abstract: MicroScale Thermophoresis (MST), an immobilization-free technology, is used to quantitate biomolecular interactions (pM-mM), ranging from protein-protein interactions to small molecule-target binding. MST, the directed movement of molecules in optically generated microscopic temperature gradients, is monitored by fluorescence. This thermophoretic movement is affected by the entropy of the hydration shell around molecules and is highly sensitive to binding reactions, which affect the size, charge, conformation, and/or hydration shell. We show how MST can be used to identify and quantify interactions between biomolecules of interest: proteins, nucleic acids, ions, etc. In one study, the binding site of a protein-protein complex is investigated with protein engineering. We also demonstrate how interactions with proteins such as GPCRs can be analyzed in a Label-Free manner using tryptophan fluorescence. With MST, affinities of membrane proteins can be probed in detergent or liposomes.

PP01.51

Spectraplakin Regulates Localization of Maternal mRNAs during Mid-Oogenesis in Drosophila

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Abstract: Spectraplakin regulates localization of maternal mRNAs during mid-oogenesis in Drosophila Jiyeon Lee and Jeongsil Kim-Ha* Spatial cues that direct the organization of the embryonic body plan or polarization of cells should be deposited during development under precise spatial and temporal control. As spatial cues for oocyte polarity, maternal mRNAs are used and become localized during oogenesis. The localization process is composed of multiple steps, but the *Drosophila* mid-oogenesis stages are notable as the time when most maternal mRNAs become localized at discrete regions of the oocyte. Microtubule rearrangement occurs during this period and is critical for the localization of axis-determining maternal mRNAs. Despite its importance, the initial event that leads to the microtubule rearrangement during mid-oogenesis is unknown. We have

identified *shot* as a key player in establishing this cytoskeletal arrangement that required for the spatial localization of axis-determining maternal mRNAs. We also found that the spatial distribution of the Shot protein is regulated by its mRNA localization. Our results suggest that the RNA localization mechanism is used not only for restricted accumulation of patterning molecules but also for the microtubule organization that leads to the initial development of oocyte polarity. *To whom correspondence should be addressed

PP01.52

Gallic Acid Abolishes the EGFR/Src/Akt/Erk-Mediated Expression of Matrix Metalloproteinase-9 in MCF-7 Breast Cancer Cells

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Abstract: In the present study, we explored the effect of gallic acid on the expression of matrix metalloproteinases (MMPs) in EGF-treated MCF-7 breast cancer cells. EGF treatment promoted cell invasion and migration, and the up-regulation of MMP-9 mRNA and protein levels. EGF treatment induced phosphorylation of EGFR and elicited Src activation, subsequently promoting Akt/NFκB (p65) and ERK/c-Jun phosphorylation in MCF-7 cells. Activation of Akt/p65 and ERK/c-Jun was responsible for the MMP-9 up-regulation in EGF-treated cells. Gallic acid repressed the EGF-induced activation of EGFR and Src; furthermore, inactivation of Akt/p65 and ERK/c-Jun was a result of the inhibitory effect of gallic acid on the EGF-induced MMP-9 up-regulation. Over-expression of constitutively active Akt and MEK1 or over-expression of constitutively active Src eradicated the inhibitory effect of gallic acid on the EGF-induced MMP-9 up-regulation. A chromosome conformation capture assay showed that EGF induced a chromosomal loop formation in the MMP-9 promoter via NFκB/p65 and AP-1/c-Jun activation. Treatment with gallic acid, EGFR inhibitor, or Src inhibitor reduced DNA looping. Taken together, our data suggest that gallic acid inhibits the activation of EGFR/Src-mediated Akt and ERK, leading to reduced levels of p65/c-Jun-mediated DNA looping and thus inhibiting MMP-9 expression in EGF-treated MCF-7 cells.

PP01.53

Abolishment of Akt/ERK-Mediated Mcl-1 Stability is Involved in Amsacrine-Induced Apoptosis of Human Leukemia U937 Cells

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Abstract: Previous studies reported that anti-cancer activity of amsacrine is attributed to its inhibitory effect on topoisomerase II. However, 9-aminoacridine derivatives, that shared the same structural scaffold with amsacrine, induced apoptosis of cancer cells through altering the expression of Bcl-2 family proteins. Thus, the goal of the present study was to explore the role of Bcl-2 family proteins in amsacrine-induced apoptosis of human leukemia U937 cells. Amsacrine-induced apoptotic death of U937 cells was characterized by caspase-9/-3 activation, increase in intracellular Ca^{2+}, mitochondrial depolarization and Mcl-1 down-regulation. Amsacrine reduced Mcl-1 protein stability, leading to Mcl-1 down-regulation. Upon exposure to amsacrine, Ca^{2+}-mediated ERK inactivation was observed in U937 cells. Meanwhile, amsacrine induced Akt degradation. Blocking of ERK-mediated Mcl-1 phosphorylation inhibited the effect of Pin1 on stabilizing Mcl-1 protein, while Akt degradation promoted GSK3b-mediated Mcl-1 degradation in amsacrine-treated cells. Restoration of ERK phosphorylation or/and Akt expression inhibited amsacrine-induced Mcl-1 down-regulation. Moreover, over-expression of Mcl-1 abrogated amsacrine-induced mitochondrial depolarization and rescued the viability of amsacrine-treated cells. Taken together, our data indicate that amsacrine promotes GSK3b-mediated Mcl-1 degradation via its inhibitory effect on Akt- and ERK-mediated Mcl-1 stability. Consequently, amsacrine induces apoptosis of U937 cells via activation of mitochondria-mediated death pathway.

PP01.54

Blue Light Irradiated Retina as a Model of Oxidative Stress-Related Retinopathies

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Abstract: Retinal exposure to short-wavelength blue light causes retinal tissue and photoreceptor damage with a significant production of reactive oxygen intermediates (ROI) in the outer retina, particularly in the rod Outer Segments (OS) [1]. Functional expression of an ectopic oxidative phosphorylation (OXPHOS) was reported in OS[2]. Here we verified whether the OXPHOS is implied in the oxidative damage caused by blue-light (BL) exposure of a mouse organotypic eye culture model. Mouse eyeball cultures were treated with BL (peak at 405 nm, output power 1 mW/cm^2) for six hours. Electron microscopy and histochemical assays showed impairment of respiratory Complexes I and II after BL exposure, both in the OS and IS. Electron transfer capacity between Complex I and II as well as activity of Complexes I and II was decreased in blue-light irradiated purified OS (BL-OS). Basal O_2 consumption and ATP synthesis were impaired in the BL-OS A severe malfunctioning of the OS aerobic respiratory capacity after BL treatment would be the consequence of a self-induced damage. An initial over-functioning of both phototransduction and respiratory chain, causing ROI production as demonstrated by cytofluorimetric analysis, would impair redox chains, perpetuating the damage and causing hypo-metabolism with eventual apoptosis of the rod. Data suggest that blue-light irradiated retina represents a good model to study oxidative stress-related retinopathies such as Age Related Macular Degeneration. 1. C. Roehlecke at al. PLoS One 8(9), e71570 2. D. Calzia, et al. Biology of the cell 105(8), 345-358

PP01.55

Spike-In RNA Variants (SIRVs) - External Transcript Isoform Controls in RNA-Seq

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Abstract: RNA spike-in controls are currently applied to assess sensitivity, input/output correlation, differential gene expression, etc. in RNA-Seq experiments. These control RNAs are monoexonic and do not represent transcript isoforms. However, the vast majority of genes in higher eukaryotes undergo alternative splicing, and transcript isoforms are present in concentrations spanning several orders of magnitude. To address this added complexity of transcriptomes, we have designed Spike-In RNA Variants (SIRVs) for the quantification of mRNA isoforms in Next Generation Sequencing (NGS). The initial consideration was to produce sets of transcripts which are variants of a given gene to provide for the training and evaluation of bioinformatics algorithms to accurately quantify, map and assemble isoforms. We have developed 7 transcript variant sets, based on human gene structures but with artificial sequences. For each of the genes, 6-18 transcript variants were derived either from known, annotated isoforms or additionally designed to comprehensively address alternative splicing, alternative transcription start and end sites, overlapping genes and antisense transcription. The SIRVs are designed to mimic human transcripts closely in terms of length (190-2500 nt) and GC content (30-51%), and the GT-AG exon-intron junction rule was observed. They do not show significant sequence similarities to any sequenced genome or transcriptome when searched against the NCBI database. Therefore, they can be spiked into total RNA from any sequenced organism also alongside existing ERCC spike-in mixes and are unambiguously identifiable in the resulting mRNA-Seq NGS data. Here, the designs of the SIRVs transcripts, mixes and spiked reference RNA samples are described.

PP01.56

Highly Efficient Gene Expression Profiling by 3′ RNA-Seq

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Abstract: RNA-Seq has been established as the new standard for transcriptome analysis. QuantSeq provides a technology to prepare highly strand-specific NGS libraries directed towards to the 3′ end of polyadenylated RNAs within 4.5 h. It requires only low input ranging from 0.1–500 ng of total RNA and enables a high degree of multiplexing. To evaluate differential gene expression with the QuantSeq protocol, FDA's Sequencing Quality Control (SEQC) standard samples A and B were used, and the detection of differential gene expression (true-positive versus false-positive) was assessed on the level of ERCC spike-in transcripts. QuantSeq showed a better performance than standard RNA-Seq while the numbers of detected ERCC transcripts stayed constant between the two methods, even when down-sampling from 10 M to 0.625 M reads. When comparing the coverages of QuantSeq to standard mRNA-Seq, a 12-fold reduction in read depth was calculated for QuantSeq, since this method only covers the 3′ end of transcripts. With a strand specificity of >99.9 %, the quantification of both antisense transcripts and overlapping genes is possible. The input-output Spearman correlation of 0.973 and 0.986 for ERCC mix 1 and mix 2 emphasizes QuantSeq's very high gene count accuracy. Data analysis is simplified since no junction detection is necessary and transcript abundances are given by read counts. Six QuantSeq datasets were aligned in only 35 min using Bowtie2 while the corresponding mRNA-Seq alignment took 2h 50min requiring TopHat2. Therefore, QuantSeq is the method of choice for gene expression analysis allowing for a fast and efficient library preparation.

PP01.57

Mincle-Mediated Translational Regulation Is Required for Strong Nitric Oxide Production and Inflammation Resolution

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Abstract: In response to persistent mycobacteria infection, the host induces a granuloma, which often fails to eradicate bacteria and results in tissue damage. Diverse host receptors are required to control the formation and resolution of granuloma, but little is known concerning their regulatory interactions. Here we show that Mincle, the inducible receptor for mycobacterial cord factor, is the key switch for the transition of macrophages from cytokine expression to high nitric oxide production. In addition to its stimulatory role on TLR-mediated transcription, Mincle enhanced the translation of key genes required for nitric oxide synthesis through p38 and eIF5A hypusination, leading to granuloma resolution. Thus, Mincle has dual functions in the promotion and subsequent resolution of inflammation during anti-mycobacterial defense using both transcriptional and translational controls.

PP01.58

T Helper Subset Cell Activation and ACAD Dedicated by Peptidylarginine Deiminase 2

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Abstract: Peptidylarginine deiminase type 2 (PADI2) is a post-translational modification enzyme that catalyzes arginine residues into the citrulline residues. Previous studies have shown that PADI2 promotes protein citrullinations in lymphocytes and it might play an important role in cell-mediated inflammation. Whether does PADI2 participate in the pathway of activated T cell autonomous death (ACAD) is still curious. In this delicate PADI2-mediated

ACAD study, we found that overexpression of PADI2 displayed higher levels of citrullinated protein which would induce the ER stresses significantly. The high levels of citrullinated protein results in unfolding protein response (UPR) of ER stresses and increases the huge protein degradation loading. Herein, PADI2 could enhance autophagy in Jurkat T cells and lead to a degradation of p62 and the accumulation of LC3-II, BCEN1, ATG5 and ATG12. Autophagy and apoptosis are two critical mechanisms both which participate against cellular stresses and decide T cell activation, survival and immuno-homeostasis. PADI2-overexpressed Jurkat T cells caused the activation of Th17 cells due to the increase mRNA expression of cytokines, such as IL-17, IL-21, IL-22 and TNFa. Cytokines declined autophagy, provoked caspase cascade expression, and led to ACAD by IL-6 shRNA inspection. Simultaneously, autophagic BCEN1 could reduce Bcl-xL expression, increase caspase cascade and cause to cell insults. Knockdown of BCEN1 might rescue T cell activation, increase cytokine release and induce ACAD. We suggested that PADI2 participated in ACAD through triggering ER stress pathway coupling with regulating autophagic processing, and stimulating Th17 activation and the expression of cytokines by PADI2-citrullinating mechanism.

PP01.59

Differentiation and Function of Mouse Endothelial Colony Forming Cells Are Inhibited by RUNX3

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Abstract: Endothelial colony forming cells (ECFCs) are progenitor cells to be committed and differentiated into mature endothelial cells. Runt-related transcription factor 3 (RUNX3) belongs to Runt family protein and is required for differentiation of specific immune cells and neurons. RUNX3 has been known that it is frequently silenced by epigenetic mechanisms or deleted and plays a role as a tumor suppressor in apoptosis and cell cycle arrest in a variety of cancers. However, the role of Runx3 in differentiation of ECFCs is not yet investigated. To determine the role of RUNX3 in differentiation of ECFC, adult bone marrow (BM)-derived hematopoietic stem cells (HSC) were isolated from RUNX3 heterozygous (Rx3+/-) or wild type (WT) mice. Differentiation of ECFC in RUNX3 heterozygous (Rx3+/-) mice was significantly increased compared to WT mice determined by number of small or large colony forming unit. Migration and tube formation ability of Rx3+/- ECFCs were significantly increased compare to WT ECFCs. Number of circulating ECFCs (CD34+/VEGFR2+ cells) were also increased in Rx+/- mice. These results indicate that haploinsufficiency of RUNX3 increases ability of ECFC differentiation and vascular forming ability. Expression level of *VEGF*, *VEGFR2*, *SDF-1*, *CXCR4* and *endothelial nitric oxide synthase (eNOS)* mRNA and protein was significantly up-regulated in Rx3+/- ECFCs compared to WT ECFCs. Finally, we found that recovery of blood flow was highly increased in Rx3+/- mice determined by hindlimb ischemia model. Taken together, our study revealed that a novel function of RUNX3 is anti-vascular forming ability by inhibition of ECFC differentiation and mobilization.

PP01.60

Effect of Orlistat and Garlic on Antioxidant Enzymes Lipid Profile of High Fat Diet Induced Obese Sprague-Dawley Rats.

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Abstract: The global prevalence of obesity is on the increase; hence the development of a safer and more effective anti-obesity therapeutic approach is becoming more of an imperative. Objective: The purpose of the current study was to investigate the effect of Orlistat and Garlic on the body weight, antioxidant properties, lipid profile of high fat diet induced obese Sprague-Dawley rats. Method: Forty Sprague-Dawley rats weighing (170 4 5 g), were divided into two groups. Group A was fed with standard chow diet while group B was fed with standard chow and Fat Emulsion to induce obesity. After 30 days, the animals in group B were divided into four groups (8 rats each) and treated with Orlistat drug (5mg/kg body weight), Garlic extract (5ml) and a combination of both for 5 weeks. Result: Treatment with Orlistat (5mg/kg), Garlic extract (5ml) and a combination of both significantly reduced the lipid profile level and body weight with Garlic having a more significant ($P<0.05$) effect. However, treatment significantly ($P<0.05$) ameliorated hepatic and renal alterations as observed in High Fat Diet treated groups. Serum ALT, AST, LDL, Triglycerides and Total cholesterol were significantly ($P<0.05$) higher in the high fat group compared with normal controls; and administration of Orlistat, Garlic extract and a combination of both significantly decreased the effect of the High Fat Diet.

PP01.61

Cardiovascular Protective Properties of Phylanthus Acidus Fruit Extracts and Fractions

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Abstract: The intake of vitamin C, carotenoids, flavonoids, and other fruit-derived antioxidants have been known to give a preventive effect against cardiovascular disease. The purpose of the present study was to investigate potential cardiovascular protective properties of fruits extracts and fractions by analyzing the antihypercholesterolemic, antioxidant, and cytotoxic activities. We screened antihypercholesterolemic activities of acetone and methanol extracts of six underutilized fruits from Indonesia based on the HMG CoA reductase inhibition activities. Those fruits were *Baccaurea racemosa* (Reinw. Ex. Blume) Mull. Arg., *Mangifera caesia* Jack, *Pouteria campechiana* (Kunth) Baehni, *Phyllanthus acidus* (L.) Skeel, *Sandoricum koetjape* (Burm. F.) Merr., and *Syzigium cumini* (L.) Skeel. The samples were separated between raw and ripe fruits and also between fruit rind, fruit pulp, and seed. The highest antihypercholesterolemic activity was exhibited by *P. acidus* ripe fruit acetone extract (inhibition 80.00%). Subsequently, we carried out successive fractionation of *P. acidus* extract using dichloromethane, ethyl acetate, and water. Then, we investigated those fractions potential for cardiovascular protective properties by

analyzing antihypercholesterol, antioxidant, and cytotoxic activities. Ethyl acetate fraction of *P. acidus* acetone extract gave the highest inhibition on HMG CoA reductase activity (inhibition 87.30%). Aqueous fraction of *P. acidus* acetone extract showed the strongest antioxidant activity by DPPH-radical scavenging activity (IC_{50} 26.06 mg/mL). Cytotoxic activity was also observed using Brine Shrimp Lethality Test (BSLT) and aqueous fraction of *P. acidus* acetone extract exhibited the strongest activity (LC_{50} 473.26 mg/mL). Based on our results, *P. acidus* fruit have potential cardiovascular protective properties.

PP01.62

Cell Localization and Sex-Related Expression of Chloride/Formate Exchanger (Cfex/Slc26a6) in Rat Organs

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Abstract: The chloride/formate exchanger Slc26a6/Cfex transports chloride, bicarbonate, oxalate, formate and hydroxyl ions in mammalian organs. In contrast to mice, Cfex expression in rat organs has been poorly studied. So, we investigated the cell localization and possible sex-related expression of Cfex in various rat organs at mRNA and protein level by quantitative RT-PCR (qRT-PCR), immunofluorescence analysis (IFC) and western blotting (WB), respectively. Specificity of the anti-CFEX antibody (CFEX-Ab) was verified by IFC in HEK293 cells transiently transfected with *rCfex* cDNA; the CFEX-Ab strongly stained the plasma membrane of *rCfex*-transfected HEK293 cells, whereas this staining was not observed in mock-transfected cells. By IFC, the Cfex protein was localized in the brush-border membrane of renal proximal tubules (S1~S2>S3) and intestinal enterocytes (duodenum>jejunum, ileum was negative), canalicular membrane of hepatocytes and apical domain of pancreatic ducts. WB analysis of total cell membranes isolated from respective organs detected a single protein band of ~120 kDa whose density matched the IFC findings. The *Cfex* mRNA expression roughly followed protein expression. Sex-dependent expression of Cfex at mRNA (qRT-PCR) and protein (WB/IFC) level was exclusively detected in kidneys of adult rats (males>females), whereas in pre-pubertal rats its expression was low and sex-independent. Furthermore, renal Cfex protein expression was downregulated by castration and unaffected by ovariectomy. Finally, testosterone upregulated, while estrogen and progesterone had no effect on renal Cfex protein expression in castrated males. Thus, Cfex is expressed in various rat organs, with male-dominant expression in kidneys that appears after puberty due to androgen stimulation.Funded by Croatian Science Foundation-project#1481.

PP01.63

Umbilical Cord Blood Mesenchymal Stem Cells Undergo Profound Metabolic Changes from Preterm to Term

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Abstract: Mesenchymal stem cell (MSC) isolated from umbilical cord (UC) are considered an excellent candidate for cell therapy in several diseases. However, little is known about MSC metabolism during their development. Therefore, we have evaluated the energy metabolism of human MSC isolated and expanded from UC of preterm (Gestational age (GA): 26, 28, 33, 35, 36 weeks) and term (GA: 39 weeks) newborns, using MSC from adult bone marrow as control. The results show that MSC at 28 weeks of GA display a high glycolysis flux, devoted to lactate fermentation; this asset switches to the oxidative phosphorylation (OXPHOS) around the 34th week of gestation. These functional data are associated with an increment of the expression of OXPHOS proteins, i.e. COX II, a subunit of Complex IV, and β subunit of ATP synthase increase thrice during the maturation from preterm (GA 28) to term (GA 39). Also the expression of Opa1, Drp1 and Fis1, proteins involved in the mitochondrial fission/fusion processes increases with the same rate, suggesting that also mitochondria undergo a maturation process. Moreover, oxidative stress status increase parallel with the gestational age, as demonstrated by the twice increment of malondialdehyde concentration and the expression of SOD2 from 28 to 39 weeks of GA. The message of this study is threefold: 1) there is a switch in MSC metabolism during the gestational development, which is linked to 2) the maturation of the respiratory apparatus of the cell, suggesting that 3) also mitochondria undergo important structural changes from preterm to adult life.

PP01.64

Transcription of Exogenous RNA in Human Platelets Using Nanoliposomes

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Abstract: Transcribing exogenous RNA in eukaryotic cells requires efficient gene delivery to the nuclei of cells, limiting the potential scope of gene therapy and synthetic biology. Manipulating gene expression in anucleate cells, such as platelets, is particularly challenging. We hypothesize that these challenges can be overcome by delivering nanoliposomes containing all the components necessary for transcription to cells, allowing for extranucleate transcription. We encapsulated a reporter DNA template, T7 RNA polymerase, and ribonucleotides within 200nm liposomes, and delivered these to *ex vivo* human platelets. This led to the internalization of nanoliposomes and accumulation of functional reporter mRNA within platelets. Internalization of RNA-transcription liposomes was measured using various

techniques, including flow cytometry, fluorescence microscopy, and quantitative PCR. By photo-caging a component of the transcription reaction, transcription within liposomes and platelets could be suppressed and controllably initiated upon irradiation with light. This initial proof-of-concept study represents a method to alter eukaryotic gene expression without gene delivery to nuclei. To our knowledge, this is the first report of direct delivery and *de novo* synthesis of mRNA to human platelets, which may have important applications in studying platelet biology and for therapeutic use. Future studies will explore the use of therapeutically relevant gene targets, as well as the ability of these liposomes to modify the gene expression of other eukaryotic cells.

PP01.65

ECE-1c Phosphorylation and Its Role in Colon Cancer Invasion

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Abstract: Background: Endothelin Converting Enzyme-1c (ECE-1c) is a metalloprotease that synthesizes endothelin-1 which may promotes cancer progression. Protein kinase CK2 regulates several cellular processes, most of them with key importance in cancer, included colon cancer. We have previously demonstrated that ECE-1c is phosphorylated by CK2. In this work, we evaluated whether CK2 prevents ECE-1c degradation, as well as its implication in colon cancer cell migration and invasion**. Material and Methods:** Protein stability was measured with the cycloheximide assay in CHO-K1 cells using TBB as a CK2 specific inhibitor. Ubiquitination was evaluated by pull-down assay in CHO-K1 cells overexpressing His-tagged Ubiquitin. Migration and invasion were analyzed in DLD-1 colon cancer cells by both transwell and matrigel assays, respectively. **Results:** (1) Inhibition of CK2 promoted ECE-1c degradation. (2) CK2-dependent phosphorylation of ECE-1c improved the migratory and invasiveness capabilities of colon cancer cells. **Conclusions**: CK2-dependent phosphorylation at the N-terminal end of ECE-1c promotes its stability, which improves migration and invasion of colon cancer cells. Acknowledgements: CONICYT Ph.D. Fellowship #21120181 (I.N.G); Grants FONDECYT #1120132 and #1160889 (J.C.T.)

PP01.66

Impact of Harungana Madagascariensis on Haematological and Hepatic Lipid Synthesis Genes Expression in Anemic Rats

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Abstract: Medicinal plants are a source of lead compounds in drug discovery. *Harungana madagascariensis* plant is known for its ethno-medicinal uses in the treatment of various human diseases. In view of this, the investigation of its impact on blood haematological parameters and hepatic lipid synthesis gene expression profiles in phenylhydrazine-induced anemic rat is vital. Single daily oral dose of the crude aqueous extract of *H. madagascariensis* leaves were co-administered for 28 days respectively in phenylhydrazine-induced anemic rats. The haematological parameters (haemoglobin, packed volume cell (PVC) and red blood cell counts) and the relative hepatic mRNA expression of lecithin-cholesterol acyltransferase (LCAT), paraoxonase-1 (PON-1), Scavenger Receptor Class B Type I (SCARB1) and 3-hydroxy-3-methylglutaryl-CoA Reductase (HMGCR) were analyzed by real time polymerase chain reaction (PCR) and electrophoresis techniques. The results showed that co-administration of aqueous extract of *H. madagascariensis* leaves significantly ($p<0.05$) improve the altered haematological parameters. Also, the expression of PON1, SCARB1 and HMGCR genes was increased significantly as compared to the control. However, the LCAT expression decreased as compared to control and anemic rats. Thus, the overall results showed that co-administration of aqueous extract of *H. madagascariensis* leaves protect against phenylhydrazine hepatotoxicity.

PP01.67

Insulin Sensitivity and Its Signaling Pathway in Pregnancy

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Abstract: Introduction. Metabolic and hormonal demands in pregnancy decrease insulin sensitivity. There is little information about insulin signaling proteins in glucose homeostasis regulating tissues during this period. Identification of insulin resistance mechanisms during pregnancy is important given its role in the pathophysiology of gestational diabetes. **Aim**: To determine changes in insulin signaling pathway in pregnancy. **Methodology**: Two groups of C57BL / 6J mice, pregnant and no pregnant, were tested for insulin sensitivity by insulin tolerance test. The abundance of active forms of insulin signaling proteins: AKT, PDK-1 and GSK-3β, as well as PTEN, an inhibitory protein of this transduction pathway, were analyzed in the liver, muscle and adipose tissue after 30 minutes of insulin induction (i.p: 1 U/kg). **Results**: We found that, compared with non-pregnant animals, insulin tolerance was decreased on day 17 of gestation. Protein immunoblotting analysis revealed that the active forms of AKT, PDK1, GSK3-β and PTEN were decreased in the liver, whereas in muscle only AKT and PDK-1 were diminished. Compared to liver and muscle, in the adipose tissue, the response to insulin-induced AKT and PDK-1 phosphorylation was lower, and show to vary between individuals. As well, no changes in the protein abundance of active PTEN and GSK-3β were observed. **Conclusion:** The active form expression of insulin signaling transduction proteins varies between insulin sensitive tissues. The adipose tissue variability between individuals could lead to the identification of markers involved in the individuals' susceptibility in the development of gestational diabetes.

PP01.68

Structural and Functional Investigation on Human Melanocortin-4 Receptor and Disease Causing Mutants

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Abstract: The melanocortin receptors (MCRs) are members of the G protein-coupled receptor (GPCR) 1 superfamily with seven transmembrane (TM). Melanocortin-4 receptor (MC4R) has been highlighted recently by genetic studies in obese humans. Previous studies have shown that extracellular (ecto) domain is related to obesity disease. Extracellular domain of human MC4R (hMC4R) has critical region for interacting with hMC4R ligand. We observed specific interactions between hMC4R ecto-domain and SHU9119, which is well known potent antagonist of hMC4R. Antagonist, SHU9119 binds to Val2, Arg7, Trp16, Asn17, Leu23 and Lys33 residues of hMC4R ecto-domain in 200mM DPC micelle. In addition, mutations in the MC4R gene are the most frequent monogenic causes of severe obesity and are described as heterozygous with loss of function. We performed NMR studies on TM2 domain of MC4R and Asp90 mutant in a micelle environment. Data shows that TM2 of MC4R forms a long α-helix with a kink at Gly98.
Interestingly, the disease-related mutant also has an α-helical conformation with a kink; however, the thermal stability and homogeneity of MC4R mutant are dramatically different from those of wild-type. The structure from molecular modeling and biochemical data suggests that Asp90 plays a key role in allosteric sodium ion binding. Our data concludes that the dynamic nature of MC4R and the sodium ion interaction in the allosteric pocket of receptor molecule together with antagonist binding are essential to its function, explaining the loss of function of the MC4R mutant.

PP01.69

Salmonella Fights a SUMO-Dependent Battle for Intracellular Survival: A New Paradigm in Host-Pathogen Crosstalk

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Abstract: *Salmonella* Typhimurium (ST) induced gastroenteritis in humans is attributed to a battery of secreted effector proteins encoded by its two *Salmonella* Pathogenicity Islands (SPI I and SPI II). These effectors orchestrate epithelial invasion and bacterial uptake into early endosomes that eventually matures into *Salmonella* containing vacuole (SCV). Once inside the SCV, ST, as reported by our recent work, deterred a key host post-translational modification process i.e. SUMOylation. Additionally perturbation of the host SUMOylation status significantly hindered the intracellular life of ST. However, the role of SUMOylation in SCV biology is poorly understood. To delineate this process in greater detail, a comparative SUMO proteome analysis of control and ST infected cells was carried out. Proteome data revealed host endocytic pathway proteins essentially Rab7a, Rab1 and Rab14 getting differentially regulated upon infection. Further *in silico* analysis highlighted the presence of multiple SUMO and SIM motif in several host and ST proteins. Interestingly, SifA, a SPI II encoded ST effector known to regulate Rab7 dependent SCV maturation harbors SUMO motif and was found to be SUMOylated *in vitro*. Establishing SUMOylation of ST effector by us is the first of its kind. We also observed that SUMOylation of SifA was required for intracellular survival. Furthermore, the precise SUMOylation status of host was detrimental in recruitment of small GTPase Rab7 a process that controls SCV maturation. Taken together these findings open a novel theme of SUMOylation dependent regulation of vesicular transport system in intracellular life of ST during infection.

PP01.70

Gene Expression of Lipoprotein Metabolism and Haematological Parameters in Anemic Wister Rats with Parquentina Nigrescens

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Abstract: The use of medicinal plants as food supplements and in the treatment of specific diseases dates back to antiquities. This study was designed to evaluate the gene expression of lipoprotein metabolism pathway and hematological parameters in phenylhydrazine-induced Wister rats co-treated with crude aqueous extract of *Parquentina nigrescens* leaves for 28 days. Relative hepatic mRNA expression of the lipoprotein metabolism genes: Lecithin-cholesterol acyltransferase (LCAT), Paraoxonase-1 (PON1), Scavenger Receptor Class B Type I (SCARB1) and 3-hydroxy-3-methylglutaryl-CoA Reductase (HMGCR) were monitored by real time polymerase chain reaction (PCR) and electrophoresis techniques. The whole blood was analyzed for haemoglobin count, percentage packed cell volume (PVC) and red blood cell counts. While the expression of SCARB1 reduced in both the induced-phenylhydrazine anemia and co-treated *P. nigrescens* extract rats, the expression of LCAT and PON1 genes were activated significantly ($p<0.05$) as compared with the control groups. However, there was a reduced level of expression of hepatic HMGCR compared with the induced-phenylhydrazine rats. The co-administration of *P. nigrescens* significantly ($p<0.05$) reversed the reduction caused by phenylhydrazine in hemoglobin, PVC and red blood cell counts. Therefore, co-administration of aqueous *P. nigrescens* leaves extract causes a hepatoprotective effect through enhancing the hepatic gene expressions of lipoprotein metabolism.

PP01.71

Stimulation of MPK38-Dependent ASK1, TGF-Beta, and p53 Signaling Pathways by p21

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Abstract: Murine protein serine-threonine kinase 38 (MPK38), also known as maternal embryonic leucine zipper kinase (MELK), is a member of the AMP-activated protein kinase (AMPK)-related kinase family and has also been implicated in various cellular processes, including cell cycle regulation, proliferation, migration, apoptosis, carcinogenesis, and signal transduction pathways. Here, we show that p21 functions as an activator of MPK38 through direct interaction. The physical association between MPK38 and p21 is mediated through the carboxyl-terminal region of MPK38 and the CDK binding region (46-71 aa) of p21 and increased in response to ASK1/TGF-beta/p53 signals. p21 phosphorylation at Thr^{55} by MPK38 implies a potential role for p21 phosphorylation in MPK38 activity regulation. Overexpression of wild-type p21, but not the p21 mutant T55A, stimulates MPK38-mediated ASK1, TGF-beta, and p53 activity by stabilizing MPK38. Consistently, p21 knockdown by small interfering RNAs displays an opposite trend, leading to the inhibition of MPK38-mediated ASK1, TGF-beta, and p53 activity. There is also a considerable inhibition of MPK38-mediated ASK1, TGF-beta, and p53 signaling activation in embryonic fibroblasts taken from p21-deficient mice. In this work, we present evidence that cyclin-dependent protein kinase inhibitor p21 can work as an *in vivo* activator of MPK38, probably contributing to the control of metabolic disorders associated with ASK1, TGF-beta, and p53 signaling.

PP01.72

Perlman Syndrome Nuclease DIS3L2 Shapes Cytoplasmic Non-Coding Transcriptome

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Abstract: The exosome-independent exoribonuclease DIS3L2 is mutated in Perlman syndrome. Here we used extensive global transcriptomic and targeted biochemical analyses to identify novel DIS3L2 substrates in human cells. We show that DIS3L2 regulates pol II transcripts, comprising selected canonical and histone-coding mRNAs, and a novel FTL_short RNA from the ferritin mRNA 5′ UTR. Importantly, DIS3L2 contributes to surveillance of pre-snRNAs during their cytoplasmic maturation. Among pol III transcripts, DIS3L2 particularly targets vault and Y RNAs and an Alu-like element BC200 RNA, but not Alu repeats, which are removed by exosome-associated DIS3. Using 3′ RACE-Seq, we demonstrate that all novel DIS3L2 substrates are uridylated *in vivo* by TUT4/TUT7 poly(U) polymerases. Uridylation-dependent DIS3L2-mediated decay can be recapitulated *in vitro*, thus reinforcing the tight cooperation between DIS3L2 and TUTases. Together these results indicate that catalytically inactive DIS3L2, characteristic of Perlman syndrome, can lead to deregulation of its target RNAs to disturb transcriptome homeostasis.

PP01.73

Caged Isoproterenol Binding to Nuclear β-Adrenergic Receptors Increases Nuclear Ca2+ Levels

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Abstract: We previously identified $ß_1$- and $ß_3$-adrenergic receptors (ß-ARs) on cardiomyocyte nuclear membranes. At the cell surface, these receptors mobilize a cascade of signalling proteins including PKA and its Ca^{2+}-modulating targets, ultimately contributing to Ca^{2+} release and alterations in cardiomyocyte contractility. The aim of the present study was to determine if nuclear ß-ARs are involved in regulating the concentration of Ca^{2+} inside the nucleus ($[Ca^{2+}]_n$). To selectively activate nuclear ß-ARs we used a cell-permeable, UV-activated, caged isoproterenol analog (cIso). Adult rat cardiomyocytes were loaded with cIso and the cell-permeable Ca^{2+} dye Fluo-4 AM. Cellular Fluo-4 fluorescence was measured before, during, and after intracellular photolysis of cIso. Upon photolysis, cIso increased $[Ca^{2+}]_n$ in adult rat cardiomyocytes. Pre-treatment with EEDQ, which alkylates and inactivates receptors on cell surface, did not block the ability of an intracellular release of Iso to increase $[Ca^{2+}]_n$, confirming that intracellular receptors regulate the $[Ca^{2+}]_n$. Loading cardiomyocytes with ryanodine or a caged analog of a ß-AR antagonist, propranolol, inhibited the increase in$[Ca^{2+}]_n$ by cIso. Inhibiting G_i with pertussis toxin, which inhibits the Iso-induced increase in transcription initiation in isolated nuclei, failed to block the ability of intracellular Iso to increase $[Ca^{2+}]_n$. An IP_3 receptor inhibitor, 2-APB, had only a partial inhibitory effect on the cIso-induced increase in $[Ca^{2+}]_n$. In contrast, pre-treating cardiomyocytes with either PKA (PKI(14-22)) or PKG (KT5823) inhibitors prevented cIso from increasing $[Ca^{2+}]_n$. These results suggest that, in adult cardiomyocytes, nuclear ß-AR activation causes an increase in $[Ca^{2+}]_n$ that requires activation of both PKA and PKG.

PP01.74

In Vitro Antiplasmodial and Antioxidant Activities of a Nigerian Multi-Herbal Mixture (Agbo-Iba)

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Abstract: Malaria, a pathogenic infectious disease results in over 1 million deaths annually. In view of the success of quinine and artemisinin, medicinal plants may well prove to be relevant sources of chemotherapeutic agents for combating the malaria scourge. *Agbo-iba*, a decoction of a number of herbal plants

used for the local management of malaria in Nigeria appears to be one of such. In this study, the phytochemical constituents, *in vitro* antioxidant and antiplasmodial properties of its ethanol extract were assessed. Phytochemical screening test revealed the presence of flavonoids, tannins, cardiac glycosides, terpenoids, alkaloids and saponins. Although the ascorbic acid standard had a significantly higher ($p < 0.05$) reducing potential, *Agbo-iba* had a significantly higher ($p < 0.05$) DPPH's radical scavenging activity (IC_{50} = 0.04 4 0.02 ×g/mL) than ascorbic acid (IC_{50} = 1.12 4 0.23×g/mL), a significantly higher ($p < 0.05$) ferric reducing antioxidant potential (198.83 4 9.46×mole Fe (II)/g extract) as compared to the ascorbic acid standard (109.17411.57×mole Fe (II)/g extract) and a 99.84% ability of inhibiting lipid peroxidation. Phytochemical quantification showed that *Agbo-iba* has high levels of total phenol, flavonoids and proanthocyanidin. The *in vitro* antiplasmodial study was done using the parasite growth inhibition assay. The ability of the multi-herbal extract to inhibit parasite growth by 50% (IC_{50} = 5.16 ng/mL) was comparable to that of the chloroquine standard (IC_{50} = 1.44 ng/mL). The high antioxidative capacity of *Agbo-iba* may be due to its rich phytochemical constituents which may also account for its antimalarial activity.

PP01.75

Sirtuins-Mediated Protein Acylation in the Regulation of Mitochondrial Function and the MAMs Organization during Adipogenesis

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Abstract: SIRT3 and SIRT5-mediated protein acylation (e.g., deacetylation, demalonylation and desuccinylation) is related to metabolic regulation and pathogenesis of obesity and type 2 diabetes (T2D). Recent studies indicated that disruption of the integrity of mitochondria-associated endoplasmic reticulum membranes (MAMs), which are defined as the contact sites between mitochondrial outer membrane and the ER membrane, is also related to T2D. In light of these observations, we investigated how SIRT3 and SIRT5 affect the differentiation and function of adipocytes through the regulation of MAMs formation and intracellular Ca^{2+} homeostasis. First, we observed a dramatic change in the amounts of these two proteins and the protein acylation profile during adipogenic differentiation of adipose-derived human mesenchymal stem cells (ad-hMSCs). To examine the roles of SIRT3 and SIRT5-mediated protein acylation in adipogenesis, we performed knockdown of SIRT3 and overexpression of SIRT5, respectively, during adipogenesis of ad-hMSCs. The results showed that these two manipulations resulted in defects in mitochondrial function and adipogenesis. Moreover, the expression of proteins that are involved in the organization and function of MAMs were upregulated and the formation of MAMs was also increased after adipogenic differentiation of 3T3-L1 preadipocytes. The findings suggest that the communications between mitochondria and ER may be regulated by sirtuin-mediated protein acylation, which plays a role in the maintenance of Ca^{2+} homeostasis in adipocytes. The observations from this and previous studies suggest that sirtuins play a role in the pathogenesis of T2D, and this scenario may help us develop novel therapies by improving the organization and function of MAMs.

PP01.76

Methylphenidate Induces Neurons and Astrocytes Loss in Hippocampus and Behavior Changes in Juvenile Rat

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Abstract: In the present study, we investigate biochemical and histochemical alterations in the hippocampus, as well as assessment the performance of juvenile rats chronically treated with methylphenidate (MPH) in behavioral tasks. In this study, Wistar rats received intraperitoneal injections of MPH (2.0 mg/kg) or an equivalent volume of 0.9% saline solution (controls), once a day, from the 15th to the 45th day of age. Twenty-four hours after the last administration of MPH the rats were decapitated (for biochemical studies), or perfused (for histochemical studies) or subjected to the behavioral tasks. Student's t test was used to evaluate the different parameters after the dates presented a normal distribution in Shapiro–Wilk test. We showed that chronic MPH treatment promoted a loss of astrocytes and neurons in hippocampus of juvenile rats. BDNF and pTrkB immunocontents, and NGF levels were decreased; while TNF-α and IL-6 levels, Iba-1 and caspase 3 cleaved immunocontents (active microglia marker and active apoptosis marker, respectively) were increased. ERK and PKCaMII signaling pathways, but not Akt and GSK-3β were decreased. We also observed that SNAP-25 was decreased by MPH treatment, while GAP-43 and synaptophisyn were not altered. Exploratory activity and memory of object recognition were impaired by MPH treatment. These findings provide additional evidence that early-life exposure to MPH can have complex effects, as well as provide new basis for understanding of the biochemical and behavioral consequences associated with chronic use of MPH during the development of central nervous system.

PP01.77

Proteomic Approach of Serum in Depression Diagnosis

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Abstract: Major depressive disorder (MDD) is a common and severe mental disorder, which can cause a considerable degree

of disability to the individual and society. Molecular mechanism of depression has been poorly understood and there has been no biological marker which can aid for the diagnosis and treatment of depression. Therefore, this study attempted to find diagnostic marker in depression by proteomic analysis of serum. It has evaluated how difference serum in MDD and healthy control affects cell survival. Viability, apoptosis and necrosis were analyzed. Then, altered proteome in serum by mood status were identified using proteomic approach. It suggested that the occurrence of disease leads to the changed environment in the serum which affects the cell viability and late apoptosis/necrosis. The peripheral alternation in the depression suggested the toxicity inside the serum and the cellular resilience which does not properly function. And then, each patient's protein expression was individually analyzed to identify biomarker candidates with accurate diagnostic ability. Finally, the protein expression was validated in MDD patients with matched normal control subjects. Here, it suggested that 3 proteins may serve as potential serum biomarkers of depression and combination of promising biomarkers has more significant diagnostic value. The peripheral signal suggests the new perspective for the future studies in the diagnosis, treatment and understanding of the depression and would help the diagnosis and treatment to get improved.

PP01.78

Carvacrol Attenuates Acute Kidney Injury Induced by Cisplatin through Suppression of MAPK and PI3K/Akt Pathways

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Abstract: Mechanisms of renoprotective effects of carvacrol against cisplatin (CP)-induced kidney injury were investigated. Male BALB/cN mice were orally gavaged with 5, 10 and 20 mg carvacrol/kg body weight for two days, 48 h after CP (13mg/kg) intraperitoneal injection. Four days after CP administration, the increase in serum creatinine and blood urea nitrogen levels coincided with histopathological findings of kidney injury. Renal oxidative stress was evidenced by increased expression of cytochrome P450 E1 (CYP2E1) and heme oxygenase-1 (HO-1). CP treatment increased the expression of phosphorylated nuclear factor-kappaB (NF-κB) p65 and tumor necrosis factor-alpha (TNF-α) in the kidneys, suggesting inflammatory response. CP intoxication induced apoptosis and inhibition of the cell cycle in the kidneys by increasing the expression of p53, p21, Bax, and caspase-3 and suppressing Bcl-2 and cyclin D1 expression. The increase in proliferating cell nuclear antigen (PCNA) suggested enhancement of DNA repair process. CP administration also resulted in activation of extracellular signal-regulated kinases 1 and 2 (ERK1/2), Akt and signal transducer and activator of transcription (STAT) 3. All these changes were dose-dependently restored by carvacrol. *In vitro*, carvacrol at concentration of 550 ×M showed a significant cytotoxicity against HeLa cells, which occured through ERK1/2 inhibition. Interestingly, carvacrol seems to protect HeLa cells treated by CP. The results of the current study suggest that carvacrol attenuated CP-induced acute renal injury by suppressing oxidative stress, apoptosis and inflammation through the mechanisms which involve modulation of PI3K/Akt and ERK/STAT3 pathways. However, some concerns arose concerning its cytoprotection against CP-treated tumor cells *in vitro*.

PP01.79

Novel Insights of Nitric Oxide Signaling: The Importance of Red Blood Cells Activity in Twin Neonates

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Abstract: Introduction: Nitric oxide (NO), synthesized by nitric oxide synthases, is an essential signaling molecule involved in many physiological processes. However, NO is a Janus-faced molecule, since it also participates in the generation of strong oxidant, peroxynitrite anion (ONOO-), in the present of elevated level of the free radical superoxide anion (O2˙-). Pregnancy, associated with an enhanced metabolism and demand for O2, may lead to the overproduction of O2˙- and other free radicals. The NO production by the umbilical cord endothelial- and the circulating red blood cells (RBCs) during pregnancy is a critical parameter for the development of oxidative stress. Oxidative stress during pregnancy may cause fetal growth restriction, complicated by intrauterine hypoxia and an impaired blood flow to the fetus. **Aim:** Our main goal was to follow the redox status of the umbilical cord RBCs during multiple pregnancies. **Results:** The number of eNOS positive RBCs from healthy, singleton pregnancies represent about 6% of the RBC population and this number was more than two times higher in twin neonates. An elevated levels of ONOO- production and damages of macromolecules; protein nitration and lipid peroxidation, were also detected in twin RBCs and this phenotype highly correlated with increased eNOS production. Additionally, the increased free radical/oxidant production was paralleled with down regulation of the expression of genes coding for antioxidant defense molecules. **Conclusion:** Twin pregnancy itself constitutes an extra challenge for the developing fetuses, the measured antioxidant parameters are at lower levels, while free radical production is significantly higher than in singletons.

PP01.80

Kigelia Africana Extract Exhibits Anti-Cancer Effect in Highly Metastatic Breast Cancer Cells Through Regulation of VCAM-1

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Abstract: The Kigelia plant has a long history of use by rural and African countries particularly for medicinal properties. Especially, Kigelia africana is an interesting example of a plant. It has been

used in traditional medicine for many years and known to exhibit several pharmacological activities, including anti-tumor effect. Virtually all patients who die from cancer including breast cancer have metastatic disease. Cancer metastasis is a complex process involving the coordinated cellular responses of both cancer cells and normal cells such as endothelial cells (ECs). Herein, we investigated the anti-cancer effect of Kigelia africana (Lam.) extract focusing on anti-metastatic effect in highly metastatic breast cancer cells MDA-MB-231. Methanol extract of Kigelia Africana (MKA) showed no cytotoxicity both on MDA-MB-231 breast cancer cells and ECs at the doses of 10, 50, 100, 200 ×g/ml. MKA significantly reduced the expression of vascular cell adhesion molecule-1 (VCAM-1), but not of intracellular adhesion molecule-1 (ICAM-1), on MDA-MB-231 cells as well as TNF-a-stimulated ECs from 50 ×g/ml. In addition, MKA dose-dependently inhibited the adhesion of MDA-MB-231 to ECs and MDA-MB-231 invasion through EC-matrigel-coated transwell membranes. These results suggest that MKA inhibits metastasis by regulating the adhesion of MDA-MB-231 to ECs and the invasion of MDA-MB-231 through ECs by inhibiting VCAM-1, providing evidence that Kigelia Africana might have a potential to be used for anti-cancer therapy.

PP01.81

Impact of Hypoxia on the Response of Chondrosarcomas to Cisplatin

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Abstract: Chondrosarcoma (CHS) is a rare bone tumor, resistant to radio- and chemotherapy. The resistance may be explained by the hypoxic feature of these tumors. However, few studies have been conducted on the involvement of hypoxia in resistance to treatments in CHS. Here, we focused on the response of chondrosarcoma to cisplatin under hypoxic conditions. Four common cell lines derived from human chondrosarcomas have been used. Survival curves were established after cisplatin treatment under normoxia (21% O_2) and hypoxia (1% O_2). Cell cycle was determined by flow cytometry and apoptosis was estimated through PARP cleavage by Western-Blotting. STAT3 phosphorylation was also evaluated by Western-Blotting. Intracellular concentration of platinum was measured by mass absorption spectrophotometry. Finally, whole exome sequencing of the four cell lines was carried out. Hypoxia increased resistance to cisplatin in only one CHS line. This resistance to cisplatin upon hypoxia was associated to a reduction of apoptosis. To understand the mechanisms involved, we evaluated the intracellular concentration of platinum but no significant difference was observed. Phosphorylation of STAT3 increases under hypoxia after cisplatin treatment in the resistant line. We analysed the whole exome sequencing and determined 205 genes mutated only in the cisplatin resistant line. The functional identification of these genes is ongoing. In conclusion, under hypoxia, the response to cisplatin is variable and depends on CHS line. Transcriptomic analysis is in progress to identify genes differentially regulated by hypoxia after cisplatin treatment in the different CHS lines.

PP01.82

An Oral Quinoline Derivative Causes the Mitotic Arrest and Overcomes Sirolimus-Resistant of Human Acute Leukemic Cells

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Abstract: Leukemia is a genetically heterogeneous malignancies and the sixth leading cause of cancer death in the United States of America. Despite great advances in the treatment of acute leukemia, a renaissance of current chemotherapy needs to be improved. The present study elucidates the underlying mechanism of a new synthetic quinoline derivative, MPT0B392 (B392) against acute leukemia. B392 causes mitotic arrest and ultimately leads to apoptosis. It was further demonstrated to be a novel microtubule-depolymerizing agent. The effects of oral administration of B392 were compared with those after intravenous administration of vincristine in an *in vivo* xenograft model, and the former was found to have a potent anti-leukemia activity. Further investigation revealed that B392 triggered induction of the mitotic spindle checkpoint, followed by mitochondrial membrane potential loss and caspases cleavage by activation of c-Jun N-terminal kinase (JNK). In addition, B392 enhanced the cytotoxicity of sirolimus in sirolimus-resistant acute leukemic cells. Combination treatment with sirolimus and B392 dramatically decreased AKT/mammalian target of rapamycin (mTOR) cascades and pro-survival myeloid cell leukemia (Mcl)-1 level compared to sirolimus treatment alone. Taken together, B392 has potential as a mitotic drug and adjunct treatment for acute leukemia, especially in sirolimus-resistant cells.

PP01.83

β-Glucosidase 2 (GBA2) Mutants in Cerebellar Ataxia/ Spastic Paraplegia Are Enzymatically Inactive and Abnormally Structured

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Abstract: Patients presenting with a combination of spastic paraplegia and cerebellar ataxia (SPastic Gait locus #46, SPG46) carry mutations in the *GBA2* gene, which encodes β-glucosidase 2, an enzyme that degrades the membrane lipid glucosylceramide. SPG46 is an infantile neurodegenerative disorder, characterized by muscle weakness and spasticity in the upper and lower limbs, and cerebellar and cerebral atrophy, thin corpus callosum, peripheral neuropathy and cognitive impairment. Currently very little is known about the involvement of GBA2 in muscle tone and motor control of the legs or additional roles in the central nervous system. We have generated cDNAs coding for five truncated and five

missense GBA2 mutants found in SPG46 patients, and expressed those mutants in cultured cells. We found that all of the disease associated GBA2 mutants were enzymatically inactive, and they had different levels of expression. Further, compared to wild-type GBA2, nine of the ten GBA2 mutants migrated very differently on native protein gels, indicating that the conformations of the mutant proteins were different from the wild-type one. The only exception was the **G683R** mutant, which behaved in a similar way as the wild-type GBA2. Remarkably, patients carrying the **G683R** mutant have milder phenotypes. These results suggest that the more severe pathology of SPG46 is due to the absence of the native molecular form of GBA2. Now we are aiming to make additional models of SPG46 in cultured cells and in Zebrafish to analyze the effect of GBA2 mutations on the development of the central and peripheral nervous system.

PP01.84

Antitumor Effect of the Novel Dual HDAC and Hsp90 Inhibitor TMUP0308 in Human Acute Myeloid Leukemia Cells

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Abstract: Acute myeloid leukemia (ALL) is the most common malignancy that occurs in children. To date, chemotherapy is the main treatment of acute leukemia, which progresses quickly if not treats properly. The failure of chemotherapy and generation of multiple drug resistance that associated with abnormal expressions of HDAC and Hsp90 in cancers. Herein, we investigated the anticancer mechanism of a new dual HDAC and Hsp90 inhibitor, TMUP0308, in human acute leukemia. In our study, the HDACs and Hsp90 were overexpressed in acute leukemia cell lines, HL-60 and MOLT-4, when compared with normal PBMC cells (peripheral blood mononuclear cell). TMUP0308 can inhibit pan-HDAC and HDAC isoforms activities; in addition, it also exhibited a stronger binding affinity to Hsp90 than 17-AAG. We further confirmed that Hsp70, acetyl-a-tubulin and acetyl-Histone H3 expressions were regulated in time- and dose-dependent manners after TMUP0308 treatment. Moreover, TMUP0308 caused cells arrest in G_2 phase followed by activation of caspases and PARP cleavage, which leading to apoptosis. Previous studies reported that over-activation of the Akt/mTOR, MAPK and JAK/STAT pathways promote cancer cells survival and anti-apoptosis. The decreases of Akt/mTOR, MAPK and JAK/STAT protein levels were also observed after leukemia cells treatment with TMUP0308. *In vivo* xenograft model, TMUP0308 obviously reduced tumor volume and without weight loss. Furthermore, our immunohistochemistry showed Hsp70 and acetyl-Histone H3 expressions increased in TMUP0308 treatment group. In conclusion, we suggest that TMUP0308 can inhibit Akt, MEK, ERK and STAT proteins downregulation though inhibition of HDACs and Hsp90 activity, ultimately triggering leukemic cells apoptosis.

PP01.85

Drug Sensitivity Screening in Chronic Lymphatic Leukemia (CLL) and Multiple Myeloma (MM) for Personalized Cancer Therapy.

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Abstract: Chronic Lymphocytic Leukemia (CLL), Multiple Myeloma and Follicular lymphoma (FL) are currently considered incurable. Although current treatment regimens prolong life for patients, CLL and MM cancer eventually relapse. Current challenges in using therapeutics against CLL and MM includes design of optimal treatment for individual patients based on characterization the tumor and its intratumor heterogeneity as observed by whole genome sequencing. Efficient therapies require a personalized approach that combines targeting lymphoma cells and the tumor microenvironment by restoring the patient's own anti-tumor immunity. To introduce individualized treatment for patients against available therapies, we aim to use a cell-based assay to define drugs that inhibit cancer cell growth. We aim to perform drug sensitivity screening to select potential drug candidates and pathway inhibitors through an approach where we directly assess patient samples. Selected drug candidates will first be validated by bioassays and flowcytometry to assess effects on intracellular mitogenic pathways (phosphoflow-based approach). We propose to use the validate candidates for xenografting and "n-of-one" clinical trial studies. The "n-of-one" protocols are organized with diagnostically based patient stratification to individualized treatments (n=1), but where each patient serves as his/her own control, and the protocol serves to test the strategy and algorithm for stratification.

PP01.86

Diabetic Human miRNA Profiles Induce Heart Disease in Mice

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Abstract: Diabetes mellitus type 2 is an increasing health problem associated with major adverse consequences for human health. MicroRNAs (miRNAs), small endogenous non-coding RNAs, regulate the expression of genes that play roles in human body via posttranscriptional inhibition. To identify the miRNAs and their target genes involved in Insulin resistance, we isolated exosomes from human blood and determined miRNA profiles. We identified a total of 3 miRNAs from 384 miRNA-target interaction pairs. Although the miRNAs regulate several different genes, we found that ulk1 gene is commonly regulated among the genes. To study the role of ULK1, we produced cardiac specific ulk1 KO mice and confirmed that they had sinus nodal dysfunction. Our study provides a bioinformatics basis for further research of molecular mechanism in Diabetes.

PP01.87

Translocating Loops Regulate the Mechanochemical Coupling and Power Production in a AAA+ Protease Motor

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Abstract: ATP-dependent proteases are crucial to maintain cellular protein homeostasis. Although previous single-molecule studies of ClpXP have shown that this motor displays alternating dwells and bursts phases during translocation, the processes underlying them remain unknown. Here, we perform a series of experiments with optical tweezers and ATPase assays to show in unprecedented detail where and when each chemical transition of the ATP-hydrolysis cycle occurs within the dwell/burst cycle of ClpXP, and identify the mechanisms that govern each phase of its mechanochemical cycle. Specifically, we show that ATP hydrolysis and phosphate release occur during the burst while ADP release/ ATP binding during the dwell. In addition, we show that residues in the highly conserved translocating pore-loops protruding the central pore of ClpX are optimized for efficient protein translocation and unfolding: their size and nature control ClpXP power generation -i.e. work produced per unit time- and the coupling efficiency between chemical and mechanical parts of the cycle. Interestingly, we observe that the conformational resetting of the pore loops between consecutive power strokes appears to time both the dwell duration and the release of ADP. Together, our results present important new insights into how evolution has optimized AAA+ proteases for efficient protein unfolding and translocation, and show that ClpXP's mechanism deviates from other well studied molecular motors (such as the Phi29 DNA packaging motor or the F1-ATPase).

PP01.88

Identification of 6-NO2Trp Containing Proteins in SHRSP – Possible Relation to Hypertension

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Abstract: Protein nitration is a post-translational modification that is induced by reactive nitrogen species in vivo under oxidative stress conditions. We have found a novel type of protein nitration, formation of 6-nitrotryptophan (6-NO_2Trp) residues, and constructed a method to identify the position of 6-NO_2Trp in proteins by using its specific antibody and LC-ESI-MS-MS. In this study, we applied this method for stroke-prone spontaneously hypertensive rat, SHRSP, which is known to cause stroke almost 100% after 25 weeks of age in male. [Methods] Liver and serum were prepared from SHRSP (male, 18.5 weeks, n=8). 6-NO_2Trp-containing proteins were detected by western blotting using anti-6-NO_2Trp antibody after SDS-PAGE and 2D-PAGE. The immuno-positive protein bands and spots were subjected to LC-ESI-MS-MS analysis. WKY rats were used as a control. [Results] We found several positive bands and spots that appeared or increased only in serum or liver of SHRSP than that in WKY. The first important protein was apolipoprotein E in serum, which was nitrated at Trp274. This Trp has an important role to form high-density lipoprotein. The second important protein was argininosuccinate synthetase (ASS) in liver, which was nitrated at Trp179. This Trp is located at neighbor of the substrate, L-cirtrullin, binding site, therefore its nitration may affect the enzyme activity. The possible relation between the modifications and hypertension will be discussed. [Conclusion] Formation of 6-NO_2Trp in apolipoprotein E and ASS may reflect high blood pressure and a complication with that and may participate formation of the clinical conditions.

PP01.89

Physical Exercise Reduces Pyruvate Carboxylase (PCB) in the Liver of Obese Mice

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Abstract: Pyruvate carboxylase (PCB) protein has been shown great importance in the upregulation of hepatic gluconeogenesis. On the other hand, physical exercise is an effective strategy to reduce the hepatic glucose production. In the present study, we evaluated the role of chronic exercise on liver PCB content in obese rodents. Swiss mice (4 weeks old) were divided into 3 groups: Control (C) sedentary animals fed on control diet, Obese (O) sedentary animals fed on HFD and Trained Obese (TO) animals fed on HFD and submitted to the training protocol. Protocol training was carried out for 1h/ day, 5 days/week, during 8 weeks and it was performed at 60% of maximum power intensity. During the last experimental week, the insulin tolerance test (ITT) was performed. Twenty-four hours after the last physical exercise session the animals were euthanized and the liver was harvested for analysis. Mice subjected to physical exercise showed decreased body weight and increased insulin sensitivity compared to O (TO vs O). We than evaluated the liver protein kinase B (AKT) phosphorylation and PCB levels in all groups. TO group showed increased Akt-phosphorylation compared to O group (TO vs O). Furthermore, O animals showed increased PCB levels compared C group (C vs O). Nevertheless, the physical exercise was able to decrease the levels of PCB in trained obese animals (TO vs O). Therefore, we conclude that exercise acting on PCB, reduces hepatic glucose production and can collaborate to reduce type 2 diabetes development in obese mice. FAPESP grant: 2013/20293-2 and 2013/21491-2.

PP01.90

Anti-Oxidative, Anti-Melanogenic, and Anti-Obesity Activities of Endlicheria Anomala

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Abstract: *Endlicheria anomala* is a tropical evergreen naturally grown in South America and Amazon. There is no report about any biological activity of *E. anomala*. In this study, anti-oxidative, anti-melanogenic, and anti-obesity activities of *E. anomala* methanol extract (EAME) were evaluated. EAME revealed more potent radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl than ascorbic acid used as a positive control. EAME also suppressed both hydrogen peroxide-induced reactive oxygen species and lipopolysaccharide-induced nitrogen oxide effectively on RAW 264.7 cells. Anti-oxidative effect of EAME might be come from the modulation of anti-oxidative enzymes such as heme oxygenase 1, thioredoxin reductase 1, NAD(P)H dehydrogenase 1, and their upstream transcription factor, nuclear factor-E2-related factor 2. Furthermore, EAME inhibited *in vitro* DOPA oxidation and 3-isobutyl-1-methylxanthine induced melanogenesis in B16F10 cells. Its anti-melanogenic activity could be result from the inhibition of tyrosinase enzyme activity and melanogenesis related protein expression. Moreover, EAME inhibited effectively pancreatic lipase enzyme activity, insulin, dexamethasone, 3-isobutyl-1-methylxanthine-induced adipocyte differentiation and lipid accumulation on 3T3-L1 preadipocytes. Its anti-adipogenic effect was modulated by cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT)/enhancer binding proteins (C/EBP) α, C/EBPβ and peroxisome proliferator-activated receptor γ gene and protein expressions. EAME also triggered lipolysis effect on adipocytes. Taken together, these results provide important new insight that *E. anomala* possesses important biological activities including anti-oxidative, anti-melanogenic, and anti-obesity activities. Therefore it might be utilized as promising materials in the field of nutraceuticals.

PP01.91

Anti-Obesity Activity of Amomum Cardamomum in 3T3-L1 Preadipocytes

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Abstract: *Amomum cardamomum* is one of Chinese medicine resources commonly used to treat symptoms of digestive disorder and chronic gastritis. Although anti-cancer and anti-oxidative activities have been reported, anti-obesity activity was unknown. In this study, anti-obesity activity of *A. cardamomum* methanol extract (ACME) was evaluated using by pancreatic lipase enzyme inhibition assay and cell culture model system. ACME inhibited effectively pancreatic lipase enzyme activity as a dose-dependent manner. Furthermore, ACME suppressed insulin, dexamethasone, 3-isobutyl-1-methylxanthine-induced adipocyte differentiation and lipid accumulation on 3T3-L1 preadipocytes in a dose-dependent manner without cytotoxicity. Anti-adipogenic effect of ACME was modulated by cytidine-cytidine-adenosine-adenosine-thymidine (CCAAT)/enhancer binding proteins (C/EBP) α, C/EBPβ and peroxisome proliferator-activated receptor γ gene and protein expressions. Moreover, ACME triggered lipolysis effect on adipocytes. Taken together, these results provide the important new insight that *A. cardamomum* possesses anti-obesity activities such as pancreatic lipase inhibition, anti-adipogenic, and lipolysis effects. It might be utilized as promising sources in the fields of nutraceuticals related to obesity.

PP01.92

Anti-Oxidative and Anti-Cancer Effects of Treculia Africana Extract in Human Colon Adenocarcinoma HT29 Cells

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Abstract: *Treculia africana*, a breadfruit species, is native to many parts of West and Tropical Africa. The crude extract of *T. africana* has been used in the folk medicine as an anti-inflammatory agent for various ailments, such as whooping cough. The objective of this study is to evaluate the anti-oxidative and anti-cancer activities of methanol extract of *T. africana* (META), and the molecular mechanisms of its anti-cancer effects in human colon carcinoma HT29 cells. META exhibited anti-oxidative activity through DPPH radical scavenging capacity and inhibited cell growth in a dose-dependent manner in HT29 cells. META treatment induced apoptosis of HT29 cells, showing the increase of the percentage of SubG1 cells. Using Annexin V and DAPI staining, apoptotic bodies of HT29 cells were also increased by META in a dose-dependent manner. The META-mediated apoptosis was associated with the up-regulation of a death receptor FAS and Bax, and decrease of Bcl-2 expression. META-treated HT29 cells also showed the release of cytochrome C from mitochondria into the cytosol, activation of caspase-3, caspase-8, and caspase-9, and proteolytic cleavage of poly ADP-ribose polymerase (PARP). These findings suggest that META may exert the anti-cancer effect in HT29 cells by inducing apoptosis through both intrinsic and extrinsic pathways.

PP01.93

Protective Effect of 4-(3, 4-Dihydroxyphenyl)-3-Buten-2-One From Phellinus Linteus on Naproxen-Induced Gastric Antral Ulcers

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Abstract: The present study was investigated the protective effect of naturally purified 4-(3, 4-dihydroxyphenyl)-3-buten-2-one (DHP) from *Phellinus linteus* against naproxen-induced gastric antral ulcers in rats. To verify the protective effect of DHP on naproxen-induced gastric antral ulcers, various doses (1, 5, and 10 mg/kg)

of DHP were pretreated for 3 days, and then gastric damage was caused by 80 mg/kg naproxen applied for 3 days. DHP prevented naproxen-induced gastric antral ulcers in a dose-dependent manner. Especially, 10 mg/kg DHP showed the best protective effect against naproxen-induced gastric antral ulcers. Also, DHP significantly attenuated naproxen-induced lipid peroxide level in gastric mucosa and increased activities of radical scavenging enzymes, such as superoxide dismutase (SOD), catalase, and glutathione peroxidase in a dose-dependent manner. A histological examination clearly demonstrated that the gastric antral ulcer induced by naproxen nearly disappeared after the pretreatment of DHP. These results suggest that DHP can inhibit naproxen-induced gastric antral ulcers through prevention of lipid peroxidation and activation of radical scavenging enzymes.

PP01.94

Juniperus Chinensis Extract Improves Stem Cell Potency via an Increase in the Proliferation of Human Skin Cells

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Abstract: The present study was investigated that *Juniperus chinensis* extract (BBXO-1) improves stem cell potency via an increase in the proliferation of human skin cells (CCD-986SK). Treatment of BBXO-1 (1 and 5 mg/ml) for 2 days significantly increased the proliferation of CCD-986SK. Especially, 5 mg/ml BBXO-1 applied for 2 days showed the highest proliferation of CCD-986SK. A clonogenic (CFU) assay was also performed to estimate the proliferation efficiency of the BBXO-1-treated CCD-986SK. In CFU assay, 5 mg/ml BBXO-1-treated CCD-986SK had an approximately 1.5-fold increase in colony formation. BBXO-1 induced extended growth characteristics, such as enhanced telomerase activity and active migration in CCD-986SK. To identify the possible activated signaling molecules involved in active cell proliferation occurring after BBXO-1 treatment, the expression levels of proliferation-related transcription factors were assessed in CCD-986SK by RT-PCR. The results of RT-PCR analysis showed upregulation of proliferation-related transcription factors (Rex1, CDK1, CDK2, and c-Myc) and stemness genes (OCT4, SOX2, Nanog, and KLF4). To estimate the relevance of PI3K and MEK signaling pathways in cell growth of BBXO-1-treated NPCs, inhibition assays were performed with LY294002 (10 mM, a specific inhibitor of PI3K) and PD98059 (10 mM, a specific inhibitor of MEK). These results clearly showed that BBXO-1 induces the proliferation of CCD-986SK via the activation of PI3K and MEK signaling pathways and improves stem cell potency via stemness acting signals, including OCT4, SOX2, Nanog, and KLF4.

PP01.95

Transcriptome Profiling of Fezf2 Expressing Intratelencephalic Projection Neurons in the Mature Mouse Motor Cortex

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Abstract: The mature cortex hosts a hugely diverse population of pyramidal neurons. Clinically important, intratelencephalic projection neurons (IT-PNs) are critical to normal forebrain circuits. Using a *Fezf2*-GFP reporter mouse, we recently demonstrated a unique identity for *Fezf2*-positive IT-PNs in layer 5 of the motor cortex. When compared to *Fezf2*-negative IT-PNs within the same layer, these neurons display complex morphology of their apical dendrites and a unique electrophysiological phenotype. Identifying the molecular profiles that underpin these properties will be essential to our understanding of their role in the healthy brain. We developed a method, using the *Fezf2*-GFP reporter mouse, where *Fezf2*+ and *Fezf2*- IT-PNs were identified and collected by *in vivo* retrograde labelling and fluorescence activated cell sorting (FACS). Applying a novel method for low input RNA-sequencing, we have generated transcriptome profiles and identified a novel set of differentially expressed genes in these separate IT-PN types. Further bioinformatics analysis of genes upregulated in *Fezf2*+ IT-PNs identified enrichment of the EF-hand calcium-binding domain, including *ryanodine receptor 2 (Ryr2)*, which alludes to a greater importance of calcium signalling in *Fezf2*+ IT-PNs. In particular, calcium signalling is important for synaptic plasticity and dendritic spine remodelling and could be critical to the distinct dendritic morphology observed in *Fezf2*+ IT-PNs. Our dataset is the first to identify the molecular underpinnings of these unique IT-PN types. Future investigations aim to test the importance of these genes for maintaining these neurons in the mature brain and will be essential for understanding the function of IT-PNs in health and disease.

PP01.96

4-O-Methyl-Ascochlorin-Reduced C-Myc Expression Promotes Apoptosis of Leukemia Cells by an AMPK/mTOR-Dependent Pathway

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Abstract: Apoptosis and senescence of advanced tumor cells and tumor regression are induced by suppression of c-Myc expression. Here, we show that 4-O-methyl-ascochlorin (MAC), a methylated derivative of the prenyl-phenol antibiotic ascochlorin isolated from *Ascochyta viciae* fungus, promoted apoptotic cell death and downregulated c-Myc expression in various human leukemia

cells. MAC markedly induced the apoptosis and repression of c-Myc protein expression in a dose-dependent manner, whereas it did not affect the protein levels of cyclin D, Bax, Bcl-2, and Bcr-Abl. The effect of 10058-F4 (a c-Myc inhibitor) or c-Myc siRNA on apoptosis also was similar to that of MAC, suggesting that the downregulation of c-Myc expression plays a role in the apoptotic effect of MAC. Further investigation showed that MAC downregulated c-Myc by inhibiting protein synthesis. MAC promoted the phosphorylation of AMP-activated protein kinase (AMPK) and inhibited the phosphorylation of mammalian target of rapamycin (mTOR) and its target proteins, including p70S6 K and 4E-BP-1. Treatment of cells with AICAR (an AMPK activator), rapamycin (an mTOR inhibitor), or mTOR siRNA downregulated c-Myc expression and induced apoptosis to a similar extent to that of MAC. These results suggest that the effect of MAC on apoptosis induction in human leukemia cells is mediated by the suppression of c-Myc protein synthesis via an AMPK/mTOR-dependent mechanism (supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning (2014R1A2A1A11050776)).

PP01.97

Suppression of C-Myc Enhances p21WAF1/CIP1-Medicated G1 Cell Cycle Arrest Through the Modulation of ERK Phosphorylation

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Abstract: Although numerous anti-cancer agents inhibit cell cycle progression via a p53-dependent mechanism, the other gene, such as proto-oncogene c-Myc, can play key factor. In this reports, we provide evidence for the ascochlorin, an isoprenoid antibiotic, is a non-toxic anti-cancer agent that induces G1 cell cycle arrest and the induction of $p21^{WAF1/CIP1}$ via downregulation of c-Myc protein expression. Ascochlorin significantly increased the G1 arrest and expression of p53 and $p21^{WAF1/CIP1}$ and decreased c-Myc expression in HCT116. In the p53-deficient cells, ascochlorin enhanced the G1 arrest-related gene except p53 expression. We found that the transcriptional repression of c-Myc is related to $p21^{WAF1/CIP1}$ expression by ascochlorin through use of the siRNA targeted against c-Myc. Further investigation showed that ascochlorin suppressed the stabilization of c-Myc protein by inhibition of ERK/4E-BP1 phosphorylation, but did not affect the c-Myc degradation mechanism such as PI3K/Akt/GSK3β. Furthermore, the PD98059 (the ERK inhibitor) and the EGK-targeted siRNA induced G1 arrest and $p21^{WAF1/CIP1}$ expression by inhibiting the c-Myc protein expression in the p53-deficient cells. These results indicated that ascochlorin-induced G1 arrest is mainly associated with downregulation of ERK phosphorylation and c-Myc expression. Thus, we reveal a role for ascochlorin in inhibiting tumor growth via G1 arrest, and identify a novel regulatory mechanism for ERK /c-Myc (supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2015R1D1A4A01019102)).

PP01.98

Probiotics Action on Gliadin Sequences Relevant to Gluten Sensitivity

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Abstract: The Celiac disease in genetically predisposed individuals is mainly induced by specific repetitive sequences in gliadins (QPQLPYP). This autoimmune disease stems from the interaction between toxic sequences and lamina propria cells, that is relevant also to other forms of gluten sensitivity. Specific endo-esoprolinase were isolated from lactic acid bacteria, suggesting possible practical applications. The ability of some probiotics at removing "toxic" celiac sequences was investigated, at first by assessing the presence and level of endo- and eso-prolinase activity in some of the most popular probiotic bacteria. Significant activities were detected in *Lactobacillus* and *Bifidum* species, as well as in the probiotic *Escherichia coli Niessle 1917*. On the basis of prolinase data, we investigated by mass spectroscopy the removal of "toxic" sequences in gliadin. A complete disappearance of these sequences was observed only with *Escherichia coli Niessle 1917*. Among the Bifidus and Lactobacillus species, only *B. bifidum MIMBb23SG* and *L. acidophilus LA5* showed a significant decrease in the "toxic" sequences. All together, this study suggests a potential use of lactic bacteria to lower gluten response in sensitive individuals, including celiacs and gluten-sensitive.

PP01.99

Utilization of CRISPR/Cas9 Towards the Generation of a DLC1-Deficient Breast Cancer Cell Line for Therapeutic Targeting

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Abstract: Introduction: Breast cancer is the second most common cause of cancer-related deaths in Canadian women, primarily due to metastatic disease. *DLC1* functions as both a tumour- and metastasis-suppressor gene exhibiting diminished expression in up to 50% of breast cancers, thereby making *DLC1* an ideal candidate for therapeutic intervention to target both primary and metastatic disease. In this study, we developed and characterized *DLC1*-deficient and control isogenic breast cancer cell lines that can be utilized in screens to identify therapeutic targets of *DLC1*-deficient cancers. **Methods**: We have employed both CRISPR/Cas9 gene editing and shRNA gene silencing in MCF7 breast cell

lines for the generation of *DLC1*-deficient breast cell models and validation of reagents required for drug target screens. Cell lines were phenotypically and biochemically characterized to assess karyotype, growth rate and endogenous DLC1 levels. **Results**: MCF7 breast adenocarcinoma was used as our breast cancer cell model to generate isogenic *DLC1*-deficient and control proficient cell lines. MCF7 were successfully transduced with a Cas9 Lentivirus and Cas9-expressing MCF7 clones were isolated and then phenotypically and biochemically characterized. Twenty-four clones were subsequently validated for use in CRISPR experiments for the generation of *DLC1* knockout cells. Similarly, MCF7 cells were transduced with a pGIPZ shDLC1 vector to generate *DLC1* knockdown cells. **Conclusion**: This preliminary research constitutes the background work necessary to provide well-characterized cell lines that can be employed in screens for the identification of therapeutic drug targets. Further validation in pre-clinical models may provide the basis for the development of novel breast cancer therapeutics.

PP01.100

APEX-Coupled Mass Spectrometry Reveals Novel Role of Hace1 in Regulating Receptor Endocytosis

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Abstract: Background: Due to their ability to regulate many cellular processes through ubiquitination, many E3 ligases play roles in tumour suppression or initiation. One such E3 ligase is Hace1, a tumour suppressor protein that is downregulated in many cancers. Currently, Hace1 function is poorly defined, and few interactors of Hace1 have been identified. We aim to find novel interactors and substrates of Hace1 to further define its role in tumour suppression. Methods: APEX proximity labelling was coupled with SILAC-MS to identify candidate interactors of Hace1. After contaminant filtering and STRING interaction analysis, the candidate protein HGS was chosen for further study. The Hace1-HGS interaction was validated by co-IP, and domains of interaction were mapped using truncation mutants. The role of Hace1 in growth factor receptor endocytosis and stability was determined through Western blot and immunofluorescence. Identification of HGS ubiquitination sites and the role of these ubiquitination modifications is currently underway. Results: APEX-coupled SILAC-MS analysis identified 270 proteins in close proximity to Hace1. Co-IP experiments showed that HGS interacted with Hace1 through its proline-rich region. The extent of HGS ubiquitination was increased with overexpression of Hace1, but Hace1 did not affect rate of HGS degradation. After stimulation with PDGF-BB, Hace1-deficient exhibited increased PDGFRβ stability and prolonged downstream signalling. Conclusions: APEX-coupled mass spectrometry analysis on Hace1 identified its novel interactor and substrate HGS, which is a regulator of receptor endocytosis. These studies reveal a role for Hace1 in growth factor downregulation, which has relevance to cancers driven by growth factors.

PP01.101

Nuclear Transport of the Transcription Factor NIT2 from Neurospora Crassa is Mediated by Impα

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Abstract: *Neurospora crassa* is a filamentous fungus that is widely used as a model organism to study different aspects of eukaryotic cells, including the classical nuclear import pathway. The protein Importin-α (Impα) is a key piece in this process, recognizing nuclear localization sequences (NLSs) in the macromolecules that will be transported. Functional experiments showed the nuclear localization of the transcription factor NIT2 in limited nitrogen conditions. Thus, this work presents the structure of Impα from *N. crassa* (NcImpα) complexed with the potential NLS of a transcription factor (NIT2_NLS) that regulate the nitrogen metabolism in fungal organisms. The aim of this work is to characterize the interaction of a non-classical NLS (NIT2_NLS) with NcImpα. Calorimetric analysis showed a high affinity of the NIT2_NLS peptide at both binding sites of NcImpα. X-ray diffraction data were collected from a single crystal of NcImpα/NIT2_NLS using a synchrotron radiation source. The final NcImpα/NIT2_NLS structure was obtained with a resolution of 2.5 Å allowing unambiguously observation that the NIT2_NLS peptide binds at major and minor binding sites of NcImpα. However, in the structure of NcImpα/NIT2_NLS, the glutamine residue (Q921) is occupying the position P4 in the major binding site, which is a position occupied, generally, by lysine or arginine residues. In conclusion, the NIT2_NLS binds with NcImpα with high affinity. Also, the structure of the complex NcImpα/NIT2_NLS showed a differential interaction that could be a specificity of the fungal nuclear import pathway and of a non-classical NLSs.

PP01.102

Molecular Interplay Between the Dimer Interface and Substrate-Binding Site of Human Peptidylarginine Deiminase 4

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Abstract: Peptidylarginine deiminase 4 catalyzes protein-arginine to protein-citrulline so called as protein deimination or citrullination. Crystal structure of PAD4 reveals that the enzyme is a homodimer with two respective active sites in which all the catalytic and substrate-binding residues are in the same monomer. This paper provides a plausible mechanism to show how PAD4 catalysis is controlled by the molecular interplay between the dimer-interface loop (I-loop) and substrate-binding loop (S-loop). Mutagenesis

and kinetic studies reveal that two hydrophobic residues, Trp347 and Val469, are critical for binding of substrate arginine at the active site; mutation of these two residues leads to a severe loss on catalytic activities of PAD4. We also identified several hydrophobic amino acid residues at the dimer interface, Leu6, Leu279 and Val283, which are important for dimerization. Ultracentrifugation analysis of these residues clearly reveals that interruption of the hydrophobicity decreases the dimer formation and hence reduces the enzyme activities. The molecular dynamic simulations and mutagenesis studies were further performed, suggesting that the dimer interface and substrate-binding site of PAD4 which consists of S-loop and I-loop, is responsible for substrate binding and dimer stabilization. We have identified five residues, Tyr435 and R441 in the I-loop, Asp465, and Val469 in the S-loop, and Trp548, which stabilizes the I-loop through Ⓟ-Ⓟ stacking interaction with Tyr435, playing crucial roles in catalysis and dimerization of PAD4. The interplay between S-loop and I-loop is crucial for PAD4 catalysis because these two loops cooperatively stabilize the correct geometry of active site to proceed further catalysis.

PP01.103

Studies on a Novel Biomarker for Atopic Dermatitis

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Abstract: Atopic dermatitis (AD) is chronic inflammatory skin disease, which affects at least 15-30% of children and 2-10% of adults in industrialized countries. In order to prevent increase in AD, detection and treatment for AD at early stage are required, but no effective biomarker has been reported yet. We have previously reported that several proteins were specifically nitrated in epidermis of patients with AD. Thus, we focused on the nitrated proteins as a candidate of the effective biomarker for AD. In this study, we used plasma of AD patients and NC/Nga mice, which demonstrate AD like skin disorders. We used anti-3-nitrotyrosine antibody (TransGenic Inc.) and anti-6-nitrotryptophan antibody, which we have developed originally, to detect the nitrated proteins by using western blot analysis. As a result, we identified that the immunoglobulin (Ig) light chain was highly nitrated in plasma of the animal model for AD than that of control mice. In addition, LC-ESI-MS-MS analysis demonstrated that these light chains contained nitrotyrosine and nitrotryptophan. We found the presence of nitrotryptophan associated with IgG in the lesional sections of skin from NC/Nga mice through immunohistochemical staining. We next applied this finding for plasma of AD patients. We found that the content of nitrotyrosine in Ig light chain was correlated with lactate dehydrogenase, which is known as a marker for AD. These results show that the content of nitrated tyrosine and tryptophan in the Ig light chain could be a possible biomarker for AD.

PP01.104

Chemotherapy Dependent Changes in Ribosomal RNA: Mechanisms and Signaling Pathways

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Abstract: A universally accepted form of treatment for many types of cancer involves the use of cytotoxic chemotherapy. While chemotherapy has been shown to reduce the cancer burden in many patients, lack of response is common. Currently, there is no biomarker that can detect a response to chemotherapy treatment. Recently, in a clinical trial for breast cancer patients treated with epirubicin and docetaxel, a significant dose-dependent reduction in tumor ribosomal RNA (rRNA) integrity values was observed at mid-treatment, which correlated with response to treatment. To determine if the phenomenon of rRNA disruption was induced by other chemotherapy agents, we developed an *in vitro* system using ovarian and breast cancer cells. We found that multiple chemotherapy agents induced rRNA disruption. We also used drug resistant cell lines to show that rRNA disruption did not occur upon treatment with drug, indicating an association of rRNA disruption with response to chemotherapy. To understand how chemotherapy agents induce rRNA disruption, we are investigating ribosomal integrity and mechanisms of rRNA degradation. Treatment of A2780 ovarian cancer cells with docetaxel resulted in a significant reduction of some cytoplasmic ribosomal proteins (RPL3, RPL4, RPSA), while other proteins are not affected (RPL7a). A mechanism for the removal of damaged rRNA, non-functional RNA decay, is dependent on the proteasome. Using a proteasome inhibitor, bortezomib, we show that rRNA disruption in response to docetaxel treatment is not reduced, indicating that docetaxel-induced rRNA disruption is not likely a form of NRD. Other types of rRNA degradation, such as ribophagy will be investigated.

PP01.105

Zebrafish Has Two swell1 Genes and Both Are Critical for Embryogenesis

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Abstract: Leucine-rich repeat containing 8A (LRRC8A, also known as SWELL1) was recently confirmed as an essential component of volume-sensitive organic osmolyte anion channel (VSOAC), and complete loss of *SWELL1* in mouse results in increased prenatal and postnatal mortality and growth retardation. Interestingly in zebrafish, we identified two candidate genes, *swell1a* and *swell1b*, which were both highly homologous to the mammalian *SWELL1* genes. To elucidate their physiological roles during embryogenesis, we began by resolving their

expression patterns during embryonic development. Like that in the mammalians, RT-PCR showed that both candidate genes are widely expressed in the early zebrafish embryos as well as in the adult tissues, including heart, liver, eyes, brain, gill, intestine, skin and kidney. Whole-mount *in situ* hybridization showed that *swell1a* and *swell1b* transcripts were ubiquitously expressed in the embryo proper at early embryos at least up to 12 hpf. Both genes can observed in the ventricular regions of the anterior neural tube at 24 hpf, and in the heart and otic vesicle at 48 hpf embryos. In order to assess their roles in the embryonic development, morpholino oligos were used to manipulate their expressions. We demonstrated that knockdown of either candidate gene in zebrafish embryos increased early mortality and promote the prevalence of abnormal phenotypes. Taken together, we identified two *swell1* genes in zebrafish with similar expression patterns, and both of the genes play critical roles in zebrafish embryonic development.

PP01.106

Effects of Metformin on Tissue Oxidative and Dicarbonyl Stress in Transgenic SHR Rats Expressing Human C-Reactive Protein

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Abstract: Metformin is the first-line drug of choice for the treatment of type 2 diabetes because it effectively suppresses gluconeogenesis in the liver. In the current study, we tested the effects of metformin on inflammation, oxidative and dicarbonyl stress using spontaneously hypertensive rats that transgenically express human C-reactive protein (SHR-CRP). We treated 8-month-old males SHR-CRP rats with metformin mixed as part of a standard diet for 4 weeks. A corresponding untreated control group of male transgenic SHR-CRP rats were fed a standard diet without metformin. In a similar fashion, we studied a group of nontransgenic SHR treated with metformin and an untreated group of nontransgenic SHR controls. Parameters of glucose and lipid metabolism and oxidative and dicarbonyl stress were measured using standard methods. Gene expression profiles were determined using Affymetrix GeneChip Arrays. In the SHR-CRP strain, we found that metformin treatment decreased circulating levels of inflammatory response marker IL-6 while levels of human CRP remained unchanged. Metformin significantly reduced oxidative stress (levels of conjugated dienes and TBARS) and dicarbonyl stress (levels of methylglyoxal) in left ventricles, but not in kidneys. In addition, metformin treatment reduced adipose tissue lipolysis associated with human CRP. Gene expression profiling in the liver revealed deregulated genes from inflammatory and insulin signaling, AMP-activated protein kinase signaling and gluconeogenesis pathways. It can be concluded that in the presence of high levels of human CRP, metformin protects against inflammation and oxidative and dicarbonyl stress in the heart, but not in the kidney.

PP01.107

Oxysterol-Binding Protein-Related Protein 4 (ORP4) is Required for Maintenance of TGN Morphology

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Abstract: Oxysterol binding protein (OSBP) and related proteins (ORPs) constitute a 12-member gene family that mediate intracellular lipid transport and/or signaling. OSBP and ORP4 form a conserved subfamily that heterodimerize, interact with the ER-associated protein VAP and bind cholesterol, oxysterols and phosphatidylinositol 4-phosphate (PI-4P). While OSBP is known to impose sterol-dependent regulation of PI-4P and sphingolipid metabolism at ER/Golgi contact sites, ORP4 is a poorly understood positive effector of cell proliferation and survival. Our aim was to understand how ORP4 regulates cell proliferation. The full-length variant ORP4L is localized to the cytosol in cells cultured in normal serum, but relocalized to the Golgi when cells were exposed to 25-hydroxycholesterol (25-OH). Localization of ORP4 to the Golgi was not dependent on OSBP. RNAi knockdown of ORP4 in HeLa cells caused significant dispersion of the trans-Golgi network (TGN) but not the cis/medial Golgi, suggesting a TGN-specific function. However, unlike OSBP, ORP4 overexpression did not influence sphingolipid synthesis nor the Golgi pool of PI-4P detected by immunofluorescence. ORP4 also interacts with and reorganizes vimentin intermediate filaments. We discovered a putative mitogen-activated protein kinase (MAPK) phosphorylation site in the lipid binding domain (serines 762,763,766,768) of ORP4 that regulates interaction with vimentin but not the Golgi. An ORP4L phosphomimetic (ORP4L-$S_{762\text{-}768}$D) caused vimentin reorganization but the corresponding dephospho-mutant (ORP4L-$S_{762\text{-}768}$A) or wild-type ORP4L were ineffective, indicating that phosphorylation enhances vimentin reorganization. These data suggest that ORP4 may connect the vimentin cytoskeleton and Golgi apparatus to constitute a novel transport pathway that is regulated by lipids and mitogenic signals.

PP01.108

Multi-Dimensional Analyses of Protein Expression, Modifications and Interactions With High Content Antibody Microarrays

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Abstract: The Kinex™ KAM-900 series antibody microarrays permit semi-quantitative measurements of the expressions, post-translational modifications and interactions of proteins with 100 ×g or less of lysate proteins recovered from cells and tissues. These chips utilize approximately 878 pan- and/or phosphosite-specific antibodies for tracking protein kinases, phosphatases and other low abundance regulatory proteins. Multiple complementary detection strategies with the KAM chips were developed that enable high depth profiling of protein levels, phosphorylation and interactions. One method (KAM) involved the capture of *in*

vitro dye-labeled proteins. False positive signals from associated proteins in complex with the target were reduced by chemical cleavage of proteins prior to capture, which also produced more uniformity of the dye signals despite vast differences in target protein sizes. Using a dye-labeled reporter antibody in a sandwich antibody microarray format (SAM), for example with the generic phosphotyrosine-specific antibodies, allowed detection of specific covalent modifications and protein-protein interactions. Other techniques used the pIMAGO stain to visualize changes in total protein phosphorylation (PAM) or a biotinylated ATP analogue to probe for ATP binding site accessibility in captured protein kinases to determine possible states of activation and interference by inhibitors (FAM). All of these detection methods were deployed to identify biomarkers and map the actions of growth factors and other agents on the signalling proteomes of human cancer cells (e.g. A431, HeLa, MCF7). Antibody microarrays are more cost-effective, sensitive, and flexible tools than any other competing proteomics methodologies to identify perturbations in response to diverse cell and animal treatments and pathological conditions.

PP01.109

Adipose Cell NLRP3 Inflammasome Upregulation Through Calcium Sensing Receptor Activation

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Abstract: Adipose tissue (AT) dysfunction, frequently observed in obesity-related metabolic and cardiovascular alterations, is associated with a disruption in the secretory profile leading to macrophage infiltration and inflammation. The calcium sensing receptor (CaSR) is a seven transmembrane domain protein expressed in numerous tissues, including AT. We have described that CaSR activation elevates proinflammatory cytokine (TNF-α, IL1β, IL6) expression in adipose cells, linking it with AT dysfunction. On the other hand, the NLRP3 inflammasome is a signaling platform assembled in response to danger signals, leading to catalytic cleavage of caspase-1 that controls maturation and secretion of IL1β. Since NLRP3 inflammasome activation has been associated to AT inflammation and dysfunction, we studied if CaSR activation stimulates the expression of NLRP3 components in human preadipocytes. LS14 preadipocytes were treated overnight with 2×M cinacalcet (a CaSR activator). Cinacalcet stimulation enhanced mRNA expression of the inflammasome components NLRP3 (65%, P=0.004), ASC (33%, P=0.008), CASP1 (159%, P=0.0004) and the proinflammatory cytokine IL1β (62%, P<0.0001). Silencing of the CaSR gene (siRNA) inhibited the response of NLRP3 inflammasome components to cinacalcet. A positive correlation (Sperman) was observed between the responses of ASC and IL1β (r=0.42, P=0.045). Interestingly, ASC showed trends towards positive correlations with the response of inflammasome-independent cytokines CCL2 (r=0.39, P=0.066) and TNFα (r=0.41, P=0.051), whereas IL6 showed no correlation (P=0.29). These data are the first report of CaSR-dependent inflammasome activation in adipose cells. The mechanisms underlying the associations among the inflammasome components and the different cytokines need further study.

PP01.110

Acetone Extract of Angelica Sinensis Inhibits Cancer Angiogenesis Through Regulation of HIF-1a Signaling

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Abstract: Purpose: The acetone extract of root of Angelica sinensis (AE-AS) is capable of inhibiting cancer cell proliferation mainly through inducing apoptosis. Hypoxia-induced hypoxia-inducible factors-1α (HIF-1α) protein accumulation and activation is critical for promoting tumor angiogenesis and growth. This study investigated the anti-angiogenic activity of AE-AS *in vitro* and *in vivo*, and the involvement of HIF-1α/vascular endothelial growth factor (VEGF)-regulated processes. **Experimental Design:** The *in vitro* tests of AE-AS were done in hypoxic human umbilical vascular endothelial cells (HUVECs) and human bladder cancer cells (T24). The anticancer and anti-angiogenic effects of AE-AS were evaluated using a Xenograft mouse model of bladder cancer. Some molecular and cellular biological assays were performed to study the underlying mechanisms. **Results:** AE-AS treatment significantly inhibited angiogenesis *in vitro* and *in vivo* evidenced by attenuation of tube formation of HUVECs, and vasculature generation in Matrigel plug and tumors. Moreover, AE-AE markedly inhibited ROS formation, HIF-1a protein synthesis/accumulation, transcriptional activity, VEGF expression/secretion, and VEGFR2 activation in hypoxic T24 cells. The hypoxia-activated phosphorylation of PI3K/AKT/mTOR/p70S6K/4EBP-1 signaling in T24 cells was also suppressed by AE-AS. Similarly, administration of AE-AS greatly inhibited the tumor growth, and the expression of HIF-1α, VEGF, and pVEGFR2 in bladder tumors. **Conclusions:** These findings support that AE-AS may be a potential therapeutic drug in cancer patients by blocking HIF-1a/VEGF-induced tumor angiogenesis.

PP01.111

The Regulation of Calcineurin Activity in the Pathophysiology of Cancer is Organ Specific and Depends on the Stage of Disease

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Abstract: Ca^{2+}/calmodulin(CaM)-dependent protein phosphatase calcineurin is a key enzyme leading to the activation of the immune system by participating in synthesis of several cytokines via dephosphorylation and activation of NFAT (nuclear factors of activated T cells) transcription factors. Calcineurin demonstrates a bifunctional activity depends on different physiological and pathophysiologal conditions of the organism. Several studies from different laboratories indicate on involvement of calcineurin in pathophysiology of cancer. Present study has been conducted to study whether the regulation of calcineurin activity in pathophysiology of cancer depends on the stage of disease, and/or is organ specific. Calcineurin activity has been determined by the spectrofluorimetric assay in plasma and tissue samples of

the oncologic patients with different stages of the breast, ovary, uterine, cervical, colorectal and gastric cancer. The plasma of healthy donors and histologically checked healthy parts of remote tissue were used as a control. Results obtained suggested that, depending on the stage (I, II, III) of disease, calcineurin activity was shown to increase in the plasma and tumor tissue of patients with ovary, colorectal and gastric cancer. In contrary, enzyme activity was shown to decrease in plasma and tumor tissue of patients with uterine, cevical and breast cancer depending on the stage of disease. It is necessary to emphasize that changes in calcineurin activity in the pathophysiology of cancer were also organ specific. **Acknowledgment:** We thank to National Center of Oncology after V.A. Fanarjyan (Ministry of Healthcare, RA), especially the Laboratory of Clinical Pathomorphology, for provided oncological samples.

PP01.112

Luteolin Inhibits Invasive Potential through the Regulation of Cadherin Switch and MMP-9 Activity in Huh7 Cells

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Abstract: Luteolin (3′,4′,5,7-tetrahydroxyflavone), an abundant flavonoid in celery, green pepper, parsley and dandelion, showed various pharmacological capacities, such as anti-inflammatory and anti-cancer activities. In this study, anti-invasive and anti-metastatic activities induced by luteolin treatment were analyzed in Huh7 human hepatoma cell line. Cadherin switch, matrix metalloproteinase (MMP)-9 activities and their upstream signaling molecules were analyzed by western blot analysis, wound healing assay and gelatin zymography. Luteolin treatment increased E-cadherin and decreased N-cadherin expression in a dose dependent manner in accordance with inhibited cell motility. MMP-9 activity was also mitigated by luteolin treatment, which was confirmed by gelatin zymography. In addition, the activation of ERK-NFκB-Snail pathway which leads to the decline of cadherin switch was also attenuated by the pretreatment of luteolin. Consequently, luteolin mitigated the epithelial mesenchymal transition by regulation of cadherin swith and MMP-9 activities through the suppressed Snail, NFκB and ERK activations in Huh7 cells.

PP01.113

Structure-Based Design of Small Molecule Anticancer Inhibitors of MTH1

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Abstract: Cancers have dysfunctional redox regulation resulting in reactive oxygen species production, damaging both DNA and free dNTPs. Significant effort has been put forth to identify several promising candidates for the treatment and prevention of cancer i.e. inhibitors of tyrosine kinase, tubulin and topoisomerase. Inhibition of MTH1, an established anticancer target, eradicates cancer by preventing sanitation of the dNTP pool. Here we report two potent inhibitors of MTH1, namely mth1i4 and mth1i5. We designed these inhibitors of MTH1 by using a chemical lead, identified by literature search and structure-guided approach. The inhibitors, mth1i4 and mth1i5, bind to highly conserved active site of MTH1 with calculated binding affinity -9.2 and -7.4 kcal/mol, respectively. We also predicted the binding conformation of 8-oxo-dGMP, a natural byproduct of hydrolysis of 8-oxo-dGTP by MTH1, with binding affinity -6.2 kcal/mol. Compounds binding at this site inhibit the function of the MTH1 protein by stabilizing an inactive conformation. The results provide a rationale for further investigation of these compounds for therapeutic application in patients with relevant diseases.

PP01.114

Analysis of Gene Expression, Protein Interactions and Signalling Pathways Altered in PBMCs From Breast Cancer Patients

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Abstract: Based on evidence that breast cancers induce local immune dysregulation via innate immune suppression, tumorigenic inflammation, and *in situ* suppression of the adaptive cell immune response. We perform a search for studies of gene expression in human peripheral blood mononuclear cells (PBMCs) from breast cancer patients through the Dataset Browser tool of GEO website. The data set selected (GDS3952) included blood from 57 women with a diagnosis of breast cancer and 31 women with normal initial mammograms. The gene expression of PBMCs from breast cancer healthy women was compared using the GEO2R application. Next, we analyzed the interaction between the gene encoding proteins with the on-line tool String and Cytoscape program. Then, through the site DAVID Bioinformatics Resources the signalling pathways were identified. Variations in the expression of 1914 genes in breast cancer PBMCs (adjust P<.001) and a high number of interactions between the encoding proteins (Highest confidence score=.900) were detected. The pathways better characterised were linked to the activation of MAPK (P=.002) and Wnt signalling pathways (P=.003) both related with proliferation, differentiation

and inflammation. Also, we found a downregulation of calcium signalling pathway (P=.001) activated by neurotransmitters, hormones and growth factors and focal adhesion pathways related with cell motility (P=.004). Our results suggest that PBMCs from women with breast cancer have and increased capacity of proliferation but also indicates that focal adhesion of these cells is impaired. This is an interesting discovery that could be limiting the capacity of the cells to migrate and eliminate breast tumour cells.

PP01.115

Disruption of Phosphatidylcholine Biosynthesis Inhibits Autophagy in ras-Transformed Intestinal Epithelial Cells

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Abstract: Phosphatidylcholine (PC), the major phospholipid in mammalian cells, is synthesized by the CDP-choline pathway under the control of the rate-limiting enzyme CTP:phosphocholine cytidylyltransferase alpha (CCTα). Cancer cells often have increased PC production for membrane biogenesis and cell proliferation. In this context, we showed that intestinal epithelial cells transformed with oncogenic H-ras (IEC-ras) have increased CCTα expression that was required for proliferation and survival, but the mechanism was unresolved. Autophagy, which involves extensive membrane biogenesis, has been implicated in the survival and proliferation of Ras-transformed cells. Thus we examined whether CCTα and PC synthesis is required for autophagy. RNAi silencing of CCTα in IEC-ras increased the level of p62 and LC3-II, indicative of autophagy inhibition. When IEC-ras were cultured in choline–deficient medium to limit the supply of substrate for PC synthesis, p62 and LC3-II also accumulated and cell growth was inhibited. Choline deprivation of non-malignant IEC had no effect on these parameters. Autophagy was restored in choline-deficient IEC-ras by addition of lysophosphatidylcholine, which is converted to PC. To determine which stage of autophagy was inhibited, cells were treated with chloroquine or bafilomycin to prevent autophagosome-lysosome fusion. Neither treatment altered p62 or LC3-II levels in IEC-ras, indicating that autophagy is blocked at, or prior to, autophagosome fusion with lysosomes. Collectively, our data show that inhibition of PC synthesis blocks autophagy and IEC-ras proliferation. Limiting PC synthesis by CCTα inhibition or choline starvation could be used to block the growth of cancer cells while sparing non-proliferative, normal cells.

PP01.116

Treadmill Exercise Reduces Liver SIRT1 S-Nitrosation in Aged Mice

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Abstract: The SIRT1 deacetylase protein plays a crucial role in cellular metabolism. It is known that during aging, decreases in SIRT1 activity can contributes to development of metabolic disorders. **Purpose:** We aim to determine if the reduction in iNOS genetically (knockout) or by exercise is related to reduced S-Nitrosation and increased SIRT1 activity. **Method: "**Young" (Y) (2 months), "sedentary-aged" (O) (30 mo) "trained aged" (TO) (30 mo) and "iNOS KO aged" (iNOSKO) (30 mo) groups were submitted to a 4 weeks treadmill training (5 days/week) at the intensity of 60% of maximum power (n=5). Twenty-four hours after the last exercise bout animals were euthanized and the liver was extracted for protein analysis. The database ´genenetwork.org` was used to perform bioinformatics analysis from liver samples of bxd mice using mitochondrial biogenesis markers that are strongly correlated to iNOS gene. **Results: "**Liver analysis showed reduced levels of gluconeogenesis enzymes G6Pase and PEPCK in "Y", "TO" and "iNOSKO" compared to "O" animals (Y, TO, iNOSKO vs O; pM0.05). We also found reduced iNOS expression, SIRT1 S-nitrosation and improved mitochondrial complexes responsible for ATP synthesis and fatty acid oxidation (MTCO1 and Uqcrc1) in both "TO" and "iNOSKO" groups compared to "Y" and "O" groups (iNOS, TO vs Y, O pM0.05). Bioinformatics analysis shown that bxd iNOS mRNA correlates inversely to mitochondrial biogenesis markers. **Conclusion:** Chronic physical exercise promotes attenuated expression of iNOS and S-nitrosation of SIRT1 contributing to increased activity of this protein, improving liver function in aged mice. Supported by FAPESP (2013/20293-2 and 2013/21491-2).

PP01.117

The Proline Isomerase FKBP25 Regulates the Nucleolar Response to Genotoxic Stress

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Abstract: The nucleolus is a vital component in the cellular response to various forms of stress, including DNA damage. Beyond simply coupling stress signaling with rRNA transcription, this organelle imparts control over the spatial organization of stress response proteins, including those involved in cell cycle regulation, apoptosis, RNA metabolism, and DNA repair. While cross-talk between nucleolar protein dynamics and DNA damage repair is well established, the regulatory mechanisms involved remain unclear. Our research has identified a novel function for the chromatin associated proline isomerase FKBP25 in mediating the nucleolar response to genotoxic stress. We have discovered

that a fraction of FKBP25 localizes to the nucleolus and interacts with dozens of ribonucleoproteins and DNA double strand break repair factors. We show that FKBP25 is required for DNA double strand break repair by homologous recombination, however it is not directly recruited to sites of breaks instead accumulating in the nucleolus. This evidence provides the first description of a proline isomerase acting in the nucleolus to mediate the repair of DNA double strand breaks. The ability of a cell to respond to DNA lesions is critical to prevent genomic instability, a hallmark of most cancers. We have identified a novel enzyme in this processes and are poised to uncover details of the molecular mechanisms of a fundamental, yet often overlooked, aspect of genomic maintenance.

PP01.118

2-HG Induces Angiogenic Activity in Bovine Aortic Endothelial Cells and Zebrafish Embryos

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Abstract: The isocitrate dehydrogenase 1 (IDH1) and 2 (IDH2) are enzymes that convert isocitrate to α-ketoglutarate (α-KG) in TCA cycle and locates in the cytoplasm (IDH1) and the mitochondria (IDH2). High level of 2-HG, which is formed by IDH 1/2 mutants from α-KG, and IDH 1/2 mutations are discovered in various cancers including glioblastomas. 2-HG stabilized hypoxia inducible factor-1α (HIF-1α), a key transcription factor, which increases the expression of genes required in metabolic adaptation, angiogenesis and metastasis in cancers under hypoxic conditions. Altered metabolism has been attributed to tumorigenesis and resistance to cancer therapies. However, the role of 2-HG in angiogenesis and vascular endothelial cells is not yet determined. Here, we showed that 2-HG significantly induced cell proliferation, migration and tube formation in bovine aorta endothelial cells (BAECs). 2-HG induced activation of mitogen-activated protein kinases (MAPKs) at the similar level induced by VEGF. Activation of matrix metalloproteinase-2 (MMP2) was significantly increased in 2-HG-treated BAECs. We confirmed 2-HG induced neovascularization *in vivo,* using embryonic chick chrioallantoic membrane (CAM) assay. Moreover, 2-HG enhanced sprouting of intersegmental vessels and dorsal longitudinal anastomotic vessels at 36 hours post fertilization (hpf) and 48 hpf in developing tg(*flk*:GFP) and tg(*fli-1a*:eGFP) zebrafish embryos. All these data suggest that 2-HG strongly induced *in vivo* and *in vitro* angiogenesis through the activation of MAPK signaling pathway.

PP01.119

A Single Allele Loss of the Insulin Receptor Trafficking Protein LMBD1 Can Cause Cardiac Hypertrophy

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Abstract: Energy homeostasis is crucial in maintaining cardiovascular function. Disturbance in such balance often results in cardiovascular disease. Limb region 1 (LMBR1) domain containing 1 (*lmbrd1*) gene belongs to the family of lipocalin-1-interacting membrane receptors. Using the Cre-LoxP system, *lmbrd1* heterozygous knockout (*lmbrd1*$^{+/-}$) mice were generated to evaluate its biological significance. Phenotypically, the *lmbrd1*$^{+/-}$ mice exhibited a significant elevation of myocardial glucose uptake and increases in heart rate, cardiac muscle contractility and ventricular wall thickness. Mechanistically, plasma membrane-localized LMBD1 interacted with insulin receptor and functioned as an insulin receptor-specific trafficking protein. Through mutation analysis and phenotypic rescue experiments, it was demonstrated that LMBD1 interacted with AP-2 to facilitate a specific clathrin-mediated endocytosis of the insulin receptor. A single allele loss of *lmbrd1* gene perturbs the insulin receptor recycling pathway and resulted in an enhancement of insulin receptor signaling cascade. Collectively, *lmbrd1* heterozygous knockout results in a constitutive activation of insulin receptor-PI3K-Akt signaling and the development of cardiac hypertrophy in mice.

PP01.120

Ecdysone Signaling Interacts with the JNK Pathway to Regulate Epithelial Fusion in a Drosophila Model of Wound Healing

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Abstract: Dorsal closure of the *Drosophila* embryo is a developmental wound-healing event in which a hole in the dorsal epidermis, occupied by a transient epithelium, the amnioserosa, is closed by migration of the epidermal flanks. Sealing occurs through morphogenesis of the epidermis in coordination with that of the amnioserosa. Dorsal closure is a popular model system for genetically characterizing the signaling pathways driving epithelial fusions. We show that Dpp, a TGF-Beta superfamily protein secreted by the epidermis, triggers production of the steroid hormone ecdysone in the amnioserosa by promoting expression of the gene *spook*. Ecdysone cooperates with JNK signaling in the dorsal epidermis and amnioserosa to activate transcription of a several genes during dorsal closure. These genes vary in their expression patterns and we present evidence that this variation is at least in part determined by distinct modes of interaction between ecdysone and JNK signaling. For example, transcription of the myosin gene *zipper*, promoting morphogenesis, involves a novel form of ecdysone receptor (EcR) signaling in which EcR forms a complex with JNK-activated AP-1 at *zipper*. This form of ecdysone

signaling is similar to interactions between the estrogen receptor and AP-1 in mammals. Our results establish *Drosophila* as a tool for genetically addressing steroid hormone receptor/AP-1 interactions in regulating gene expression. Furthermore, we have shown that communication between tissues regulates morphogenesis. Similar signaling likely regulates the coordinated morphogenesis of vertebrate tissues during epithelial fusions.

PP01.121

Understanding the Roles of Numb Isoforms in Cancer Development

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Abstract: The endocytic adaptor protein Numb has a key role in development as an inhibitor of the Notch signaling pathway. Four protein isoforms of Numb are produced in vertebrates through the alternative splicing (AS) of exon 3 and exon 9. The expression of exon 9 is developmentally regulated. Exon 9 included (exon9+) isoforms are predominantly expressed in progenitor tissues, whereas exon 9 excluded (exon9-) isoforms are highly expressed in adult tissues. Analyses of AS events in multiple cancers revealed a switch in Numb isoform expression from the exon9- isoform to the exon9+ isoform, suggesting that the misregulation of AS of Numb may have a role in tumorigenesis. The project aims to investigate (1) the signaling pathways that are differentially regulated by Numb isoforms, and (2) the tumorigenesis properties of Numb isoforms in xenograft mouse models. We have knocked out Numb and deleted exon 9 independently in a number of cell lines including human breast cancer cell lines. These cell lines are under investigation for tumorigenic activities of Numb and Numb isoforms in vitro and in vivo. To this end, less activation of the mammalian Target Of Rapamycin (mTOR) signaling pathway was observed in the exon 9 deleted cell lines comparing to the parental cell lines.

PP01.122

Genetic Polymorphism Contribute to Bladder Cancer Risk in Egyptian Population

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Abstract: Background: Bladder cancer is one of the most common cancers of the urinary tractand a major problem worldwide. In Egypt, carcinoma of the bladder is the most prevalent cancer accounting for as many as 31% of all cancer cases. Genome-wide association studies thought the association between bladder cancer risk and single nucleotide polymorphism (SNP) of rs9642880 (G>T) and rs710521 (A>G), but the results remain inconclusive. **Aim**: To assess the association between SNPs rs9642880 and rs710521 genotypes and bladder cancer risk, in Egyptian patients. **Methods:** Urine samples were collected from 150 patients' confirmed having bladder cancer pathologically and 50 controls. DNA was extracted from all urine samples precipitate. By PCR and restriction fragment length polymorphism (RFLP), the genotypes of both SNPs: rs9642880 and rs710521, were identified. **Results:** The frequency distributions of the genotype pattern for the SNP rs9642880 was GG 37%, GT 48% and TT 15%; while for the SNP rs710521 was GG 9%, AG 26% and AA 65%. **Conclusion:** Our results suggest that the rs9642880 (GT/TT) and rs710521 (GA/AA) genotypes were significantly (p> 0.01) associated with bladder cancer risk. Detection of these genotypes is useful diagnostic and prognostic markers for bladder cancer risk in Egyptian population.

PP01.123

Induction of ER Stress in the Intestinal Mucosa of Crohn's Disease Patients

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Abstract: The pathogenesis of Crohn's disease (CD) is still complex and under investigation. Recent evidences suggest a link between CD with endoplasmic reticulum (ER) stress. Here we tested the hypothesis that ER stress plays a role in the pathophysiology of CD. To test this, intestinal mucosa and mesenteric adipose tissue (MAT) were collected from surgical specimens of active ileal CD patients or respective controls. ER stress markers was evaluated by immunoblotting and qPCR assays. This study was approved by the institutional ethics committee of the University of Campinas. Our results show an increased expression of sXBP1 in the intestinal mucosa of CD patients compared to controls. We also demonstrate the activation of PERK/EIF2alpha pathway in intestine of CD patients, by the increased PERK gene expression as well as EIF2alpha protein expression and pEIF2alpha/EIF2alpha ratio, however no activation in MAT. By qPCR we observed an increase in the activated form of ATF6 in the intestinal mucosa of CD patients, however no difference in protein content and in MAT. We also observed an increased expression of genes related to ER stress activation in intestinal mucosa, like ATF3, CALR, STC2, DNAJC3, GRP94 and GRP78. No differences was observed in MAT, except for chaperones modulation of GRP94 and GRP78. Our results demonstrate the activation of the three branches of ER stress in the intestinal mucosa of CD patients, while no activation in MAT. Thus, ER stress is an important mechanism of the inflammatory process in CD and may be an attractive therapeutic target.

PP01.124

Strength and Aerobic Training Prevents Hyperinsulinemia, Insulin Resistance and Inflammation in Fructose-Fed Animals

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Abstract: The aim of this study was to compare the effects of strength, and aerobic exeercise on metabolic disorders induced by fructose-rich diet. 120d old *wistar* rats were randomized into four groups (n=8–14): C (Control-diet sedentary), F (fed on a fructose-rich-diet sedentary), FA (fed on a fructose-rich-diet and subject to aerobic exercise), FS (fed on a fructose-rich-diet and subject to strength exercise). At the end of the 8-week experiment, glucose homeostasis, serum biochemistry, tissue triglycerides, serum inflammatory proteins and inflammatory pathways activation were evaluated and analyzed using a 2-way ANOVA. The database ´genenetwork.org` was used to perform bioinformatics from mRNA of bxd mice liver samples, using inflammatory markers strongly correlated with insulin pathway. At the end of experiment fructose was able to induce glucose intolerance, insulin resistance and low-grade inflammation (C vs F pM0.01). On the other hand, both physical training exercise protocols induced remarkable reduction in glucose intolerance, insulin resistance, tissue triglycerides content, systemic inflammation, and inflammatory pathways, which was achieved through c-Jun NH2-terminal kinase (JNK) phosphorylation and factor nuclear kappa B (NFkB) activation in both the liver and the muscle (F vs FA and FS pM0.01). Yet, the strength protocol exerted greater effects on insulin sensitivity, liver lipids content and inflammation than aerobic (FA vs FS $p \leq 0.05$). Our data suggest that strength training lead more robust responses in most of the disorders triggered by the fructose-rich diet concluding that strength training could be the most suitable strategy to prevent or treat different metabolic diseases. Supported by Fapesp (Grant: 2013/20293-2 and 2013/21491-2).

PP01.125

Chromatin Remodeling Factors and Epigenetic Modifications Associated With B Cell Differentiation

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Abstract: Germinal Center (GC) microenvironment is the main source of memory B cells and plasma cells that produce high-affinity antibodies necessary to protect the body against invading microorganisms. However, this beneficial aspect of GC cells is somewhat overshadowed by their detrimental role in lymphomagenesis, for the majority of B-cell lymphomas originate from GC cells. It has been shown that inactivation of CRTC2 and the repression of its target genes is required for B cell differentiation into plasma cells. However, the mechanisms of CRTC2 regulation during B cell differentiation have not been fully explored. We demonstrated that CRTC2 interacts with components of chromatin remodeling complex in GC B cells. Specifically, using Ramos B-cells we tested the CRTC2 association with BRG1, which is the central catalytic ATPase of the SWI/SNF chromatin-remodeling complex, and demonstrated that CRTC2 binds BRG1 and forms a complex on CRTC2-target promoters. Additionally, we screened some histone-modifying enzymes associated to BRG1 and CRTC2 and its correlation with epigenetic markers. Our results suggest that CRTC2, through its interaction with chromatin remodeling complex and histone-modifying enzymes, regulates B cell development.

PP01.126

Identification of Metabolic Alteration of Prostate by YY1 Using Proteomic Approach

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Abstract: The metabolic alteration is observed in most of cancer cells including prostate. Prostate has the unique metabolism that is to accumulate the zinc (Zn) of 10-fold more than other soft tissues. As prostate becomes more aggressive cancerous cells, Zn level is decreased by 80~90% in normal prostate. Ying yang 1(YY1) is known to regulate pathogenesis of prostate cancer as well as mitochondria function. The structure stabilization of YY1 depends on Zn ions. We performed proteomic analysis to identify correlation of YY1 and metabolism alteration of prostate by using each prostate cell lines: normal prostate epithelial cell lines (RWPE-1), androgen dependent prostate cancer (ADPC) cell lines (LNCaP) and androgen independent prostate cancer (AIPC) cell lines (DU145, PC3). We identified and analyzed that YY1 was regulated with proteins related to energy metabolism including tricarboxylic acid cycle (TCA) and oxidative phosphorylation. In normal prostate cell, TCA cycle is inhibited by increase of Zn level that inhibits aconitase. We speculated that YY1 influenced by Zn level may have effect on both aconitase and other proteins related to energy metabolism and consequently may alter prostate metabolism.

PP01.127

Identification of Rheumatoid Factor-Correlated Serum Proteins by Comparative Proteomic Approach

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Abstract: Rheumatoid arthritis (RA) is a chronic autoimmune disease of the joints. Rheumatoid factor (RF) is commonly used to diagnose RA. However, RF is not sufficient to confirm the diagnosis of RA. That's why diagnostic biomarkers for RA are needed to be found. In this study, we conducted the proteomic approach to find diagnostic biomarkers for RA and it is expected that RA will be diagnosed more rapidly with simple blood test. We divided the individual serum into three groups according to levels of RF. Each group's serum is pooled, and liquid chromatography-tandem mass spectrometry is used for mass measurement of peptides in the pooled serum. After then, protein is identified by using the Spectrum Mill software and is analyzed with Mass Profiler Professional (MPP) and GeneGo MetaCore v 6.15. We identified 146 proteins in normal group (<18 IU/ml) and 129 proteins in both low abnormal and high abnormal group (>54 IU/ml). We found 2-fold differences of 13 proteins between normal and abnormal group. Also, we found 2-fold differences of 6 proteins between normal and high abnormal group (*P* value <0.05). In functional analysis, proteins related with Classical and Alternative complement pathway were activated in abnormal group compared to normal group. In further study, we need to select these proteins for quantitative analysis using multiple reaction monitoring(MRM).

PP01.128

Exercise Training and Omega-3 Fattys-Acids Modulate GPR120 Expression in Liver of Obese and Insulin Resistant Mice

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Abstract: Background: GPR120 is an omega-3 fatty acids receptor with anti-inflammatory properties. However, the mechanisms responsible for regulating GPR120 expression are still not completely understood. **Objectives:** We investigated the expression of GPR120 in liver of lean and obese mice after acute physical exercise session or exercise training, as well as their impact on the insulin signaling and inflammation. **Methods:** Swiss mice were subjected to an acute physical exercise session, and than fragments of hepatic tissue were removed for GPR120 expression analysis through Western Blotting. On another experiment, mice were fed on a standard (C) or high-fat diet (HF) for 8 weeks. Subsequent to the development of obesity, part of these animals were subjected to a physical exercise training (HF+Exe), a treatment with flax seed oil (HF+FS) or both interventions at the same time (HF+Exe+FS) for another four weeks. At the end of the experiment, liver was extracted for analysis of GPR120 protein content and proteins involved in pathways of insulin and inflammation. **Results:** Acute physical exercise did not show difference in GPR120 expression (pU0.05). Nevertheless, exercise training and the treatment with flax seed were able to increase liver protein content of GPR120 of obese mice. Furthermore, HF+EXE and HF+EXE+FS groups showed decrease inflammatory proteins compared with HF group (pM0.05). **Conclusions:** Exercise training and flax seed oil seem to be able to increase protein expression of GPR120 in the liver of obese animals as well as reduce the activity of proteins involved in the inflammatory signal (Fapesp Grant: 2014/15258-6).

PP01.129

Impact of ETFDH Mutation on Bioenergetic Metabolism and Differentiation of Human Skeletal Muscle Cells

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Abstract: Multiple Acyl-CoA Dehydrogenase Deficiency (MADD) is an autosomal recessive inherited disorder of fatty acid metabolism in mitochondria. Most forms of MADD are caused by mutations in electron transfer flavoprotein (ETF) gene and ETF-dehydrogenase (ETFDH) gene that results in dysfunctional or insufficient of ETF and ETF:ubiquinone oxidoreductase (ETF:QO). ETF:QO is an important regulator in coupling fatty acid β-oxidation and electron transport chain. Lipid myopathies were shown in most MADD patients. We previously found that impaired mitochondrial function and increased lipid droplets in the lymphoblasts from the carrier and affected MADD patients. We also found the muscle fiber type switching in the affected patient. Compared to an average of 50% slow twitch fiber in normal individuals, we found that only 33% in the MADD patient. In order to clarify the role of ETFDH mutation in the muscular pathogenesis of MADD, we identified two types ETFDH of mutations, c.250G>A and ETFDH c.92C>T from the MADD patients. We constructed and transfected three types of plasmids with ETFDH variants (c.250G>A, c.92C>T, and c.250G>A/c.92C>T) and one with ETFDH wildtype into human skeletal muscle (HSkM) cells, respectively. We found higher amount of lipid droplets accumulation and abnormal myogenic differentiation in the transfected HSkM cells harboring ETFDH variants. Furthermore, we found decreased mitochondrial oxygen consumption rate and fatty acid metabolism in the transfected HSkM harboring ETFDH variants. Future work studying ETFDH mutations has great potential for contributing to our understanding the pathogenesis of MADD and therapeutic drug supplementation to avoid metabolic decompensations in the MADD adults with myopathy.

PP01.130

Activation of Pro-Angiogenic Transcription Factors and Cytokine Expression Is Modulated by the Extra-Cellular Matrix

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Abstract: Inflammation and angiogenesis are linked in many physiological processes. Human umbilical vascular endothelial cells (HUVEC) stimulated with vascular endothelial growth

factor (VEGF) or sphingosine-1-phosphate (S1P) expressed pro-inflammatory cytokines. Cytokine secretion was also found to be modulated by specific extracellular matrix (ECM) proteins. Collagen I and Fibronectin strongly induced the expression of IL-6, IL-8 and MCP-1, while Laminin I induced only IL-6 and IL-8. Collagen IV and Matrigel had no effect on expression. When cells were grown on tissue culture treated plastic coated with different ECM proteins and treated with either VEGF or S1P, we observed an additive effect in cytokine production. During proliferation assays, only Collagen I significantly increased cell proliferation; this indicated no correlation between proliferation and cytokine expression. The use of pharmacological inhibitors identified the signal transducers and activators of transcription (STAT), AP-1 and NF-kB as regulators of cytokine expression. The activation of these transcription factors by ECM, VEGF or S1P individually or in combination was further investigated using immunoprecipitation, western blot and immunofluorescence. STAT1, STAT3, STAT5 and AP-1 were found to be constitutively active in HUVEC, whereas NF-kB showed a different result and was only activated after higher doses of S1P. As STATs and AP-1 are constitutively active, NF-kB appears to be the primary regulator of cytokine production. This indicates the extracellular environment is a significant element in the expression of inflammatory cytokines.

PP01.131

In Vitro Cytotoxicity and Membrane Damage After Exposure by Morinda Citrifolia Linn Extract

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Abstract: "Noni" *Morinda citrifolia Linn* is a plant used in Brazil both as food and for therapeutic purposes such as antibacterial, analgesic, expectorant and anti-inflammatory. Since several toxicity effects already have been reported by feeding from *M. citrifolia*, toxicity profile evaluation on it is required. In this study was evaluated the cytotoxicity effect of the alcoholic extract of the *M. citrifolia* fruit and the oxidative or protective effect in erythrocyte membrane. The *M. citrifolia Linn* extract exhibited cytotoxic activities in a concentration-dependent manner after 24, 48 and 72h incubation against human leucocytes using trypan blue exclusion test and MTT assay in the concentration 2- 0.0625 mg / ml. In addition, after exposure, changes in shape of the leucocytes cell membrane were noticed. This extract showed a strong antioxidant property by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and β-carotene systems with activity higher than 65% at all concentrations tested (2- 0.0625 mg / ml). The oxidative stress in erythrocyte membrane demonstrated the highly protective effect from alcoholic extract. This extract were haemobiocompatible at concentrations below 0.0625 mg/ml, had a lower myelosuppressive effect in haematopoietic progenitor cells and showed low genotoxic effects *in vitro*, on human lymphocyte cells. Additionally, these compounds also showed low-toxicity *in vivo* as defined a LD50 > 2000 mg/kg. In this assay, we have not observed death in the animals exposed to treatment with alcoholic extract. In conclusion, we report a powerful antioxidant agent due to its protective profile, revealing a promising extract in order to prevent for oxidative damage.

PP01.132

Phospho-Ubiquitin Variants Modulate Parkin Activity

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Abstract: The E3 ubiquitin (Ub) ligase parkin directs damaged mitochondria for mitophagy by tagging outer membrane proteins with Ub chains. Parkin's role in mitophagy is crucial for neuronal tissue maintenance, causing it to be a key drug target in neurodegenerative diseases such as Parkinson's disease. Phosphorylation of Ub at Ser65 by the PTEN-induced putative kinase 1 activates parkin's ubiquitination function. The role of other Ub phosphosites as well as their associated kinases remain unknown. Using genetic code expansion, we site-specfically produced phosphorylated Ub (pUb) variants (pUb^{S57}, pUb^{S12}, pUb^{S20}, pUb^{S65}) to probe their roles in modulating parkin activity. Purification of pUb^{S57} revealed a +3 frameshifted protein (Δ7 Ub) that entirely skips the in-frame UAG codon. We were able to purify the pUb away from this novel product of mistranslation. Although pUb^{S12} and pUb^{S20} do not stimulate parkin, we observed significant activation when pUb^{S65} is the sole substrate. Decreasing the phosphorylation level of Ub^{S20} while keeping the amount of pUb^{S65} constant resulted in a dose-dependent increase in parkin activity. This highlights the importance of producing pure phosphoprotein since parkin activity is sensitive to the stoichiometry and location of phosphorylation on its substrate Ub. Further functional investigations of these Ub phosphosites will identify novel drug targets in neurodegenerative signalling networks.

PP01.133

Synthesis of Differentially Activated Pprotein Kinase B/Akt by Site-specific Incorporation of Phosphoserine

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Abstract: Protein kinase B (Akt1) is a central node in the PI3K/Akt signaling pathway that regulates cell survival. The substrate specificity of Akt1 varies depending on the phosphorylation site. It has been difficult or impossible to synthesize site-specifically phosphorylated proteins in pure form to study this phenomenon. I employ genetic code expansion strategies that overcome this bottleneck by enabling co-translational incorporation of phosphoserine (pSer) residues at any desired location of Akt1. Phosphorylated Akt1 variants are being produced using established methods by co-expressing the human gene with a plasmid containing a tRNA synthetase, tRNA and elongation factor mutant

needed to direct pSer incorporation at UAG codons. In our study human Akt1 was successfully cloned and expressed in *E. coli* BL21 (DE3) cells. Using genetic code expansion, we produced active Akt1 directly from *E. coli* for the first time (Balasuriya *et al*. 2016 in preparation) and without 3 upstream kinases and lipid second messengers that are normally needed. The biosynthesis of site-specifically phosphorylated Akt1 variants (Thr308pSer and Ser473pSer) was confirmed through western blot analysis and tandem mass spectrometry. Both variants displayed significant kinase activity with GSK-3 peptide substrate in comparison with the inactive Akt1. Since Akt1 mediated P13K signaling is over-activated in most human cancers, Akt1 is a current and highly promising drug target. Our pAkt1 variants will be used to uncover novel Akt1 substrates and inhibitors. Our work will provide new insights into molecular basis of phosphorylation signaling in the PI3K/Akt signaling pathway.

PP01.134

Paeonia Suffruticosa Inhibits Pancreatic Cancer Cells Growth

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Abstract: *Paeonia suffruticosa* (PS) has long been widely used as traditional medicine to treat various diseases. The molecular mechanisms by which PS exerts its anti-oxidant and anti-inflammatory activities are well known, but the anticancer activity is relatively not yet well understood. This study aims to investigate antitumor efficacy of water extract of PS *in vitro* and *in vivo* on pancreatic cancer cells. Results showed that PS induced inhibitory effects on PANC1 pancreatic cancer cell proliferation and migration. By screening the proangiogenic subgroup of chemokines, CXCL1 is significantly downregulated by PS. In PANC1 cells, shRNA mediated down-regulation of CXCL1 confirmed its role in inhibition of cancer cell proliferation and invasion. Addition of CXCL1 partially rescues the shRNA effects on cancer cells where reactivation of NFκB and AKT may be involved. Oral administration of PS efficiently suppressed tumorigenic growth with no adverse effects. In addition, PS may act synergistically for gemcitabine-triggered tumor growth. These results suggest that PS may be used as a safe and potent complementary and alternative therapy for patients with pancreatic cancer.

PP01.135

Suppression of Concentration-Sensitive Sodium Channel During Lipopolysaccharide-Induced Acute Lung Injury in Mice

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Abstract: Background: Concentration-sensitive sodium channels (Na_Cs) are amiloride-insensitive sodium channels expressed in alveolar type II epithelial cells and pulmonary microvascular endothelial cells in mouse lungs. The physiological role of Na_C in the lungs is uncertain. Since various ion channels, including epithelial sodium channels (ENaCs), play a role in maintaining lung fluid balance, we hypothesized that Na_C activity is downregulated during acute lung injury (ALI), which promotes formation of pulmonary edema. Methods: Lipopolysaccharide (LPS) was transtracheally administered in mouse models of ALI, and the same dose of PBS was administered in control mice. Bronchoalveolar lavage (BAL) neutrophils were counted as a marker of ALI, and lung water contents (LWCs) were measured as a marker of pulmonary edema. Na_C protein expression in lung was detected by immunoblotting and immunofluorescence. Gene expressions of Na_C and ENaC were analyzed by quantitative RT-PCR over a time course of 14 days. Results: The number of BAL neutrophils peaked on day 2 after LPS administration and returned to baseline on day 6. LWCs in LPS-administered mice gradually increased until day 8 and recovered on day 14. Na_C protein expression in the lungs of LPS-administered mice dynamically decreased from day 2 to day 6 and recovered on day 8. Na_C mRNA expression decreased in parallel with expressions of alpha-, beta-, and gamma-ENaC during ALI. Conclusion: Since Na_C expression decreased during formation of pulmonary edema and recovered in the convalescent phase of ALI, Na_Cs may contribute to alveolar fluid clearance via sodium transport similar to that of ENaCs.

PP01.136

The Roles of Low Molecular Mass Antioxidants on Oxidative Stress Biomarkers and Severity of Ischemic Stroke in Albino Rats

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Abstract: Ischemic stroke (IS) is caused by a blood clot or occluding arteries in the brain which reduces oxygen supply to specific area of the brain, resulting in rapid cell death in the core of the affected region. Excessive generation of reactive oxygen species (ROS) and impairment of endogenous antioxidant defense mechanism begins immediately after the onset of IS, resulting in secondary events leading to neuronal dysfunction and death. This study evaluated the effects of low molecular mass antioxidants, Vitamin C, Vitamin E, α-lipoic acid, dimethyl sulfoxide and mannitol in the management of IS. The stroke was induced in rats by common carotid artery occlusion. The treatment protocol included oral administration of the antioxidants for two weeks in three different doses (22.5, 45 and 67.5 mg/kg). Serum and brain tissue homogenates were assessed for oxidative stress biomarkers

(SOD, CAT, GPX activities and MDA level). The results of the study showed significant increased (P<0.05) in the activities of the antioxidant enzymes and reduction of MDA in the groups treated with LMMA in dose dependent manner. Histological examination of the brain tissues of the experimental rats also showed improvement compared with the SI non treated group. The groups treated with vitamin C, α-lipoic acid and DMSO appeared to do better than all other groups. The study concluded that LMMA reduces oxidative stress and its biomarkers in ischemic rats and underscores relevance of LMMA in the management of ischemic stroke.

PP01.137

PAR-3 and Syndecan-4 Regulate the Focal Adhesion Dynamics of Astrocytes Stimulated With Neuronal Thy-1

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Abstract: Thy-1 is a small glycoprotein expressed in the neuronal plasma membrane. We have previously shown that astrocyte adhesion, polarization and migration are induced by the interaction of Thy-1 in neurons with avb3 integrin and Syndecan-4 in astrocytes. However, the signaling mechanisms triggered downstream of Syndecan-4 remain unknown. In addition, PAR-3, an important adaptor protein that typically forms a complex with PAR-6/aPKC to induce cell polarization, reportedly, does not participate in astrocyte polarization. Therefore, we studied the participation of both Syndecan-4 and PAR-3 in astrocyte adhesion/migration induced by Thy-1. Rat DITNC-1 astrocytes were transfected with siRNA against PAR-3, Syndecan-4 or siRNA control, treated with Thy-1-Fc or an Fc control protein and then followed in wound-healing assays or video microscopy to monitor focal adhesion assembly/disassembly. Focal adhesion disassembly was also studied by pre-treating cells for 4 h with nocodazol. After drug washout, cells were stimulated for different time periods. We found that Thy-1 accelerated the disassembly of nocodazol-induced focal adhesions. Additionally, DITNC-1 cells lacking PAR-3 or Syndecan-4 and stimulated with Thy-1 migrated less and the disassembly of their focal adhesions was delayed compared to control cells. Moreover, focal adhesions were larger in cells lacking PAR-3, but smaller in Syndecan-4-deficient cells than in control cells, implicating both proteins in astrocyte adhesion induced by Thy-1. These results suggest the existence of a hitherto unexplored role for PAR-3 and Syndecan-4 in the regulation of focal adhesion turnover.
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PP01.138

Capturing Major Conformational Motions in the Folding of the Intrinsically Disordered Amyloid-β Peptide

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Abstract: Aggregation of soluble amyloid-β (Aβ) peptide into amyloid fibrils is a common form of self-assembly phenomenon that has a fundamental association with the pathology of Alzheimer's disease. Currently, it is believed that the aggregation starts with conformational changes from α-helix to β-sheet, or this so-called protein misfolding. Understanding the molecular basis of the transition from α-helix to β-sheet will shed light on Aβ's pathway to aggregational forms and their exact conformations. We investigate the molecular-level mechanics of the conformational change using molecular dynamics (MD) simulations. The strategy is to simulate from the two ends of the misfolding pathway: α-helical Aβ monomer (PDB:1IYT) and a monomeric unit of the β-sheet amyloid fibril (PDB:2BEG). A novel approach is developed to draw insight from this complex molecular process. It combines a clustering method and principal components analysis (PCA) to capture and abstract large-scale molecular motions. Sequences of structures in solutions are resolved to elucidate the main changes in the structure of the peptide. Observations of MD simulations in aqueous solution suggested that the amyloid-β peptide is intrinsically disordered, and the "misfolding process" is not a clear transition from α-helix to β-sheet. We have identified possible pre-fibrillization equilibrium structures showing an amphipathic propensity, as well as a key role of the extremities of the peptide in the nucleic seed which further conformational changes. This is consistent with the knowledge that Aβ(1-42) is more prone to fibrillization than the slightly truncated forms for 40 and 38 residues.

PP01.139

Effects of Anti-CD44 Monoclonal Antibodies on Metabolic Profiles of Acute Myeloid Leukemia

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Abstract: Acute myeloid leukemia (AML) is a heterogeneous disease characterized by accumulation of immature hematopoietic cells due to a blockage in myeloid differentiation. Ligation of CD44, with monoclonal antibodies (mAbs) could reverse the differentiation blockage of leukemic blasts in most AML subtypes. Thus rendering CD44 a promising target for AML therapy. However, its underlying molecular mechanisms have not been fully elucidated yet. In this study, we are interested in determining the metabolic changes that take place during the treatment of AML cells with the CD44-specific mAb. The state-of-the-art Nuclear Magnetic Resonance (NMR) technology was used to identify the

metabolic profile for HL60 cells and monitor the overall metabolic consequences of treatment with anti-CD44 mAbs.[1]H-NMR experiments demonstrated that anti-CD44 treatment induced considerable changes in the metabolic profiles of HL60 cell lines. A total of 23 identified metabolites were statistically significant ($p < 0.05$). Loading plots for principal component analysis revealed increase in the levels of isoleucine, valine, leucine, alanine, acetate, glutamine, succinate, myo-inositol, glycine, formate, phenylalanine, while remarkable decrease in glycerophosphocholine, phosphocholine, choline, aspartate, fumarate, malonate, and creatine were observed. These most notable responses included changes to tricarboxylic acid cycle (TCA) intermediates, including succinate and fumarate. Moreover, This was validated by dramatically reduction in succinate dehydrogenase enzyme (SDH) activity in HL60 by the treatment of anti-CD44 mAbs. Our findings demonstrate that metabolite profiles describes the actual functional state of the cells and opens new perspectives in using metabolic profiling to support the possibilities for the development of CD44-targeted therapy of AML.

PP01.140

Differential Expression of the Mini-Chromosome-Maintenance Complex of Infected C. Elegans by Orsay Virus

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Abstract: The recent discovery of a RNA (+) nodavirus in C. elegans (Orsay virus) and the establishment of a viral infection model in this organism are allowing us to study the viral immune response in C. elegans using reverse genetics. In mammalian the protein sensors that recognize foreign nucleic-acid from viral infection to mount the innate immune response use an Oligonucleotide Binding (OB) fold. In this study we determined in which extent viral infection affect the expression of any OB fold gene in the nematode using C. elegans strains containing extra chromosomal arrays of Promotor::GFP . Using confocal microscopy we determined the spatial and temporal GFP expression of each OB gene specific promotor fusion available. Our results show differential expression in the pharynx for gene specific promotors that belong to the mini chromosome maintenance complex (mcm) gene family. These proteins helicases form a protein complex (mcm) that unwinds the DNA template to license the DNA to replicate from the origin of replication. These data suggest that the orsay virus may hijack some of the components of the host DNA synthesis machinery to replicate himself and compete with the host for survival. This is consistent with other studies in mammalian showing the involvement and association of the mcm complex in viral replication of nucleic-acids for HIV, adenovirus, influenza, and cytomegalovirus.

PP01.141

Loss of Calreticulin Uncovers a Critical Role for Calcium in Regulating Cholesterol Homeostasis

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Abstract: The Endoplasmic Reticulum (ER) is a major Ca^{2+} store and a site for regulating folding quality of nascent glycoproteins. It is crucial for the ER to maintain homeostasis to ensure proper cell functioning and disruptions of ER homeostasis have been linked to many pathological and physiological disorders. Calreticulin is a Ca^{2+} buffering chaperone that resides in the ER lumen. Calreticulin deficiency (*crt-/-*) in mice is embryonic lethal due to abnormal cardiac development but can be rescued with cardiac-specific expression of activated calcineurin. The rescue mice have a functional heart but display metabolic problems like growth retardation, hypoglycemia and elevated blood cholesterol and triglycerides indicating that calreticulin may play a role in energy metabolism. The purpose of this study is to address the molecular pathway responsible for this defect in energy metabolism by investigating the role of calreticulin and ER Ca^{2+} in modulating lipid synthesis through the sterol regulatory element-binding protein (SREBP) pathway. The SREBPs are ER membrane transcription factors responsible for activating genes involved in lipid metabolism. We have found that *crt-/-* mouse embryonic fibroblasts have higher levels of cholesterol and triglyceride and an enhanced SREBP activity. Intracellular distribution of cholesterol is also affected in the absence of calreticulin and it is sensitive to changes in the ER luminal Ca^{2+}. We concluded that loss of calreticulin and changes in the ER luminal Ca^{2+} affect cholesterol distribution, SREBP processing and activation, and contributes to the metabolic syndrome observed in the *crt-/-* rescue mice.

PP01.142

PROtein FEeding in CElegans (PROFECE) a New Method to Study Gut-Microbiota Interaction During Neuro/Muscular Development

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Abstract: Similar to human, the intestine of C. elegans contain and process constantly E.coli bacteria since it fed on bacterial lawn. This observation led us to consider the bacterial lawn of C. elegans not only as a food source but as a microbiota environment that we could modify by genetic engineering to study host-pathogen interaction in the gut of the nematode. Since the orsay virus in C. elegans was recently discovered we validated this method using this viral system. In this study we developed a novel method called PROtein FEeding in C. Elegans (PROFECE) that fed the worm with protein expressed in the E. coli bacterial lawn and looked at the

effect of the viral proteins alpha and delta during development using survival assay, fluorescence microsocopy and behavioral assay with a multi-wormtracker. Survival assays shows that the viral proteins affect lifespan and provoke intestinal malfunctions and abnormalities. Finally we observed using wormtrackers that worm fed with E. coli lawn expressing different viral proteins have behavior that is similar but different from the two basics mode of locomotion roaming and dwelling observed previously when nematode recognize food. Our results suggest that nematode growing in the presence of the orsay viral proteins have an altered muscular or neuronal function.

PP01.143

Characterization of Urinary Extracellular Vesicles From Diabetic Rat

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Abstract: Extracellular vesicles (EVs) are small membrane vesicles derived from multivesicular bodies or from the plasma membrane. The cargo of EVs includes the proteins, lipids and nucleic acids of the cells from which they originate. Most cell types release EVs that then enter the bodily fluids. We observe significant changes in HK-2 cells when they were incubated with diabetic rat urine, compared to cells incubated with control rat urine. We investigated the protein components of the vesicles extracted from urine. For that, urine was collect from control and diabetic rats with/without kidney damage and EVs isolation was performed through of ultracentrifugation, and the vesicles morphology were analyzed by electronic microscopic. For clinical biomarker discovery, LC-MS based large-scale quantitative proteomic analysis was realized. Our results show a significant difference in the protein content of healthy control rat-urine extracellular vesicles regarding diabetic rat. These results suggest that urinary EVs can be a useful tool in the identification of new markers associated with diabetic nephropathy. Acknowledgements: Innova-Corfo 13IDL2-23502

PP01.144

SmcHD1 is an Important Regulator of Select Members of a Multiple Gene Family Encoding Cell Surface Receptors

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Abstract: Structural maintenance of chromosome hinge domain containing 1 (SmcHD1) was discovered as an epigenetic silencer of the inactive X chromosome in female mice. It was required for survival of both male and female mice. Later studies suggested that besides genes on the X chromosome, other types of autosomal genes including imprinted genes and clustered protocadherin genes were regulated by SmcHD1. In order to elucidate its role in gene regulation, we made anti-SmcHD1 antiserum and performed ChIP-seq. We found that SmcHD1 occupancy in intergenic regions and near the transcriptional start sites of some genes. The predicted SmcHD1 binding sites were analysed using a variety of software in order to understand the types of transcription factor binding motifs associated with SmcHD1 binding. Binding sites near genes were used for create gene ontogeny pathways to understand its function. We used 5-azacytidine to reduce global demethylation and found many DNA loci lost SmcHD1 occupancy. Finally, gene editing techniques created SmcHD1 null cells to verify SmcHD1 gene targets identified above. We found select members of a multigene family encoding cell surface receptors were dysregulated upon loss of SmcHD1. In addition loss of SmcHD1, changed cell signaling events in response to specific ligands.

PP01.145

Differential Expression of MiR-224 in Serum and Liver Tissue in Hepatocellular Carcinoma Patients

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Abstract: Background: Hepatocellular carcinoma (HCC) is the fifth most frequently diagnosed cancer in the World. MicroRNAs are crucial in cancer development and progression. MiR-224 is one of the most commonly dysregulated miRNAs in HCC. **Aim:** This study aimed to confirm whether serum miR-224 level is correlated to the liver tissues status in HCC patients and possible use as diagnostic biomarker for HCC. **Methods:** Serum samples were collected from 65 patients with HCC and 20 controls. qRT-PCR assays used to evaluate the expression level of miR-224 in serum and liver tissues. **Results:** MiR-224 was found to be down regulated in the serum of HCC patients compared to controls. The low level of serum miR-224 was correlated with higher tumor grade and fibrosis and directly reflects the changes of tumor stage. **Conclusion:** Serum miR-224 expression level can reflect the status of tumor and liver damage in HCC patients and holds great promise as a novel noninvasive biomarker.

PP01.146

Signaling Pathways Mediated Through the Adaptor Protein GADS (Grb2-Related Adaptor Downstream of Shc) That Drive Leukemia

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Abstract: There is still a critical need to understand downstream signaling pathways that drive BCR-ABL positive myeloid and lymphoid leukemia and identify alternative drug targets. The oncogenic potential of BCR-ABL relies on the constitutive activation of the ABL tyrosine kinase. The adaptor protein GRB2 plays a critical role in connecting BCR-ABL to it substrates. The auto-phosphorylation of tyrosine 177 (Y177) in BCR-ABL has been shown to be essential for its transformation potential. It leads to the SH2 domain dependent recruitment of GRB2 which through its SH3 domains bind specific proteins and bring them into a complex with BCR-ABL. GRB2 binds to GAB2 and SOS which leads to the activation of the Ras and PI3 kinase signaling pathways required for BCR-ABL-mediated transformation.
Our lab identified another adaptor protein called GADS that is hematopoietic specific and found phosphorylation of BCR-ABL on Y177 leads also to the SH2 domain dependent recruitment of GADS. Interestingly the SH3 domains of GADS and GRB2 have distinct binding specificity. We hypothesize GADS could mediate leukemia downstream of BCR-ABL through the recruitment of specific signaling intermediates. The human CML-T1 cell line was used and a GADS (*GRAP2*) knockout line was generated using the CRISPR-Cas 9-based genome editing technology. To investigate the effects of GADS expression on the BCR-ABL signaling pathways, the phosphotyrosine profiles of the parental and the GADS KO cell lines were compared using a comprehensive phosphoproteomic approach. Interestingly the regulated phosphoproteins are known to be involved in T-cell receptor signaling, in JAK/STAT signaling and in cell adhesion.

PP01.147

Fyn-Related Kinase Suppresses Epithelial-To-Mesenchymal Transition in Breast Cancer Cells via STAT3 Signaling

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Abstract: The human Fyn-related kinase (FRK) is non-receptor tyrosine kinase protein and a candidate tumor suppressor with three functional domains, namely, the Src homology 3 (SH3), SH2 and the kinase domain. FRK also possesses a C-terminal Y497 that is conserved in Src family kinases (SFKs), that putatively regulates the enzymatic activity of FRK. Our studies show that substituting tyrosine 497 with a phenylalanine residue results in the constitutive activation of FRK thereby validating the regulatory role of Y497 on the catalytic activation of FRK. FRK has been previously shown to play growth-inhibitory roles in breast cancer cells. We have demonstrated that FRK not only inhibits breast cancer cell proliferation but also cell migration *in vitro*. Through the use of high-throughput peptide arrays we identified STAT3 as a downstream signaling target of FRK and determined that the presence of wild type FRK and especially FRK Y497F (constitutively active mutant) induces the inactivation of STAT3. We have specifically demonstrated that the stable overexpression of wild-type or FRK-Y497F in the FRK-negative and highly invasive breast cancer cell line MDA-MB-231 repressed the activation STAT3, JNK and p38, leading to the transcriptional repression of E-cadherin and Slug (SNAI2), key regulators of epithelial-mesenchymal transition (EMT). Overall, we present evidence that FRK plays a role in suppressing breast cancer cell proliferation and migration potentially via the Jak/STAT pathway. These data therefore suggest that upregulating FRK may have clinical implications in the suppression breast cancer metastasis.

PP01.148

Endothelin-1 Biosynthesis in Adult Cardiac Ventricular Myocytes and Fibroblasts

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Abstract: Type B endothelin receptors (ETB) are located in the nuclear envelope of adult ventricular cardiomyocytes (ACVMs). Activation of nuclear ETB induces a transient increase in nuclear calcium, activates NO production, and inhibits transcription initiation. In both ACVMs and endothelial cells, endocytosed rhodamine-endothelin colocalized with lysotracker but was not observed at the nuclear membrane. Thus, the ligand for nuclear ETB may be endogenous endothelin. The purpose of this study was to characterize the regulation and subcellular localization of endothelin biosynthesis in ACVMs and adult cardiac fibroblasts. Endothelin-1 mRNA was detected in both cell types. Regulation of endothelin production is primarily at the level of transcription. Application of TGFβ increased ET-1 mRNA in both ACVMs and fibroblasts whereas angiotensin II was only effective in increasing ET-1 mRNA in fibroblasts. In both cell types immunocytofluorescence experiments revealed endothelin-1 immunoreactivity, comprising all stages of peptide maturation, was detected either on or near the nuclear membrane. Endothelin converting enzyme 1 (ECE1) is a metalloprotease that converts big endothelin to the biologically active 21-amino acid peptide. ECE1 immunoreactivity was associated with the T-tubules and nuclear or perinuclear membranes in ACVMs. In contrast, ECE1 immunoreactivity was observed both in the nucleus and the cytosol, but not at the plasma membrane, in passage 2-3 cardiac fibroblasts. These data suggest that endogenous endothelin may be available to activate nuclear ETB in both ACVMs and fibroblasts in response to extracellular stimuli.

PP01.149

Inhibition of Scavenger Receptor BI Suppresses Androgen Pathway Activity & Induces Cytotoxicity in Prostate Cancer Cells

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Abstract: Androgen pathway activity persists in castration-resistant prostate cancer (CRPC). A proposed mechanism for continued androgen activity is *de novo* intratumoral synthesis of androgen receptor agonists from the precursor cholesterol. The lipoprotein-cholesterol receptor, scavenger receptor BI (SRBI), is upregulated in CRPC models. We hypothesize that SRBI is a source of cholesterol for *de novo* steroidogenesis in CRPC cells using the castration-resistant LNCaP-derived cell line, C4-2. Cells were transfected with either non-targeted (NC) or stealth RNAi duplexes (Thermo Fisher) targeting SRBI to silence protein expression (SRB1-KD). Prostate specific antigen (PSA) levels in media from SRB1-KD samples were reduced to 39% compared to control (20.941.4 ng/mL/×g protein SRBI-KD vs. 34.542.4 ng/mL/×g protein NC; n=4, $p<0.05$). Intracellular testosterone concentrations measured by LC-MS showed a 2-fold reduction in SRBI-KD samples (0.20 ng/mL/mg) compared to NC (0.41 ng/mL/mg; n=1). Additionally, SRBI-KD cells exhibited reduced cell viability as measured by MTS assay and induction of G_1/S cell cycle arrest as assessed by propidium iodide cell cycle analysis (70.947.9% G_0-G_1 phase SRBI-KD vs. 58.344.1% NC; n=4, $p<0.05$). SRBI-KD cells showed elevated induction of autophagy as assessed by changes in LC3-I:LC3-II ratio and formation of autophagosomes, alongside induction of senescence as assessed by measuring senescence associated beta-galactosidase activity. Further, treating C4-2 cells with the SRB1 inhibitor, BLT-1, lead to decrease PSA secretion, and cotreatment with the cholesterol synthesis inhibitor, simvastatin, synergistically decreased PSA secretion. These results suggest that under androgen-deprived conditions, SRBI targeting can decrease androgen pathway signalling and induce cellular stress, leading to G_1/S arrest and decreased proliferation.

PP01.150

Cell Surface Calreticulin Presentation is Modified by Alpha-integrin Expression and Function

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Abstract: Calreticulin is a multifunctional protein with multiple subcellular and extracellular distributions. Its major concentration is within the endoplasmic reticulum (ER) lumen, where it acts as a chaperone protein and calcium reservoir. Immunogenic cell death is a form of apoptosis precipitated by ER-stress inducing agents, whereupon calreticulin presented on the cell surface acts as a signal for phagocytic engulfment by macrophages. Understanding the cellular mechanisms controlling surface calreticulin presentation is an important facet toward optimizing an effective immune-mediated antitumor response. Integrins mediate bi-directional signaling between the extracellular environment and intracellular processes. Prior studies have shown that calreticulin interacts with the conserved a-integrin KxGFFKR cytosolic motif. We show that cell adhesion of T-leukemic cells promote calreticulin binding to α-integrin that was correlated with enhanced chemoresistance. Furthermore, cells expressing the minimal a-integrin motif consisting only the KxGFFKR cytosolic domain exhibited constitutive survival signaling in an adhesion-independent manner, concurrent with constitutive calreticulin interaction. We show that cell surface calreticulin level is controlled by cell adhesion and by the level of integrins expressed. This leads to our proposal that **α-integrins play an important role in decreasing the immunogenicity of chemotherapy through KxGFFKR motif sequestration of calreticulin in the cytosol.** Using a calreticulin null T-cell based model, we can confirm that anthracycline-induced surface calreticulin presentation requires endogenous expression of calreticulin. Engagement of normal integrins via adhesion, or expression of the minimal KxGFFKR constitutive active motif, reduces the levels of anthracycline-stimulated surface calreticulin. Finally, surface calreticulin level is inversely correlated with the overall level of integrin expression.

PP01.151

PI3Kp110δ Drives Crohn's Disease-Like Intestinal Fibrosis in SHIP Deficient Mice

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Abstract: Crohn's disease (CD) is an immune-mediated disease characterized by inflammation along the gastrointestinal tract. One in 3 people with CD will develop intestinal fibrosis requiring surgery within 10 years of diagnosis. Despite that, there are no treatments that target intestinal fibrosis. Our laboratory reported that mice deficient in the Src homology 2 domain-containing inositol polyphosphate 5'-phosphatase ($SHIP^{-/-}$) develop spontaneous CD-like intestinal inflammation with arginase-dependent fibrosis. We also reported that increased arginase I activity in $SHIP^{-/-}$ macrophages was dependent on increased Class IA phosphatidylinositol 3-kinase (PI3K) p110δ. Based on this, we hypothesize that $SHIP^{-/-}$ mice develop fibrosis due to increased PI3Kp110δ activity. $SHIP^{-/-}$ mice were crossed with mice deficient in PI3Kp110δ activity ($PI3Kp110\delta^{DA/DA}$). $PI3Kp110\delta^{DA/DA}SHIP^{-/-}$ mice have less intestinal fibrosis than their $SHIP^{-/-}$ littermates including: reduced TGFβ, muscle thickening, $vimentin^{+}$ mesenchymal cells, collagen deposition, IL-4, IL-13, and arginase activity. PI3Kp110δ deficiency also reduced immune cell infiltration and IL-1β in $SHIP^{-/-}$ ileum suggesting that PI3Kp110δ and/or fibrosis may contribute to inflammation. $SHIP^{-/-}$ mice were also treated with a PI3Kp110δ isoform-specific inhibitor, IC87114. Inhibition of PI3Kp110δ activity also reduced the above parameters associated with intestinal fibrosis in $SHIP^{-/-}$ mice. Our data suggest that SHIP deficient mice develop intestinal fibrosis due to increased PI3Kp110δ activity. Moreover, targeting PI3Kp110δ activity may be an effective strategy to reduce intestinal fibrosis in people with CD. Importantly,

idelalisib, an PI3Kp110δ isoform-specific inhibitor, is already licensed for use in people with certain leukemias and lymphomas, so may be rapidly translatable into effective therapy for intestinal fibrosis in people with CD.

PP01.152

Oncogenic KRAS Signaling Promotes Transcriptional Upregulation of xCT to Support Tumorigenicity by Preventing ROS Overload

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Abstract: Activating mutations in KRAS are found in ~90% of pancreatic cancers, ~40% of colorectal cancers, and ~30% of NSCLC. To date no effective therapy exists for patients of this genetic subset, giving an impetus to develop novel therapeutic agents targeting downstream effectors of KRAS. Literature provides evidence that reactive oxygen species (ROS) is essential for KRAS-mediated tumorigenicity and that KRAS-driven cancer cells generate more ROS than do normal cells. Counterintuitively, we found that mouse 3T3 fibroblasts transformed with mutant-KRASG12V had lower levels of ROS, as compared to normal cells, which suggests the activation of an intrinsic antioxidant defense mechanism. Whole transcriptome microarray and qPCR revealed that xCT(SLC7A11) mRNA was differentially upregulated in KRAS-transformed cells in response to exogenous ROS stimuli. xCT is responsible for the cellular uptake of cystine, the rate-limiting precursor in the synthesis of glutathione (GSH). As such, we hypothesized that oncogenic KRAS promotes the transcription of xCT to support tumorigenicity by preventing ROS overload. To further corroborate our findings, xCT-knockout or wildtype mouse embryonic fibroblasts (MEFs) were stably transduced with KRASG12V and injected subcutaneously into nude mice. Remarkably, xCT-deficiency resulted in a three-fold reduction in the doubling time of tumor xenografts. Finally, through gene set enrichment analysis (GSEA) of our microarray data and a series of biochemical assays, we provide evidence that the upregulation of xCT is mediated by ETS-1, a transcription factor downstream of the RAS/RAF/MEK/ERK signaling cascade. Altogether, our work suggests that oncogenic KRAS signaling supports tumorigenicity by transcriptional upregulation of xCT via ETS-1.

PP01.153

Characterization of ATP9A, a Member of P4-ATPase Family of Phospholipid Flippases

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Abstract: P4-ATPases comprise a subfamily of P-type ATPases implicated in the ATP-dependent flipping of phospholipids across cell membranes. This generates and maintains transverse phospholipid asymmetry, a property important for many biological processes including vesicle trafficking. The importance of P4-ATPases is highlighted by the finding that mutations in several P4-ATPases are associated with severe human disorders. ATP9A is a P4-ATPase that is expressed in brain, retina, pancreas and other tissues. However, little is known about the biochemical properties or localization of ATP9A. Interestingly, loss of Neo1p, the yeast homologue of ATP9A, is lethal. The purpose of this study is to investigate the functional properties and cellular localization of human ATP9A in order to define its role in cell physiology and disease. Human ATP9A was expressed in HEK293T cells, purified by immunoaffinity chromatography, and reconstituted into liposomes for functional characterization. ATP9A exhibited little if any phospholipid-dependent ATPase activity, but underwent hydroxylamine-sensitive phosphorylation, a characteristic feature of the P-type ATPase reaction cycle. A monoclonal antibody to ATP9A was generated for analysis of ATP9A in cells and tissues by western blotting and immunofluorescence microscopy. Endogenous ATP9A was primarily localized to the inner segments of retina tissues. In transfected HEK293T cells ATP9A localized to perinuclear and peripheral punctate structures possibly related to the endocytic pathway. Our findings suggest that ATP9A undergoes autophosphorylation, but fails to dephosphorylate, possibly due to detergent solubilization or lack of an accessory protein. Further studies on endogenous ATP9A should provide further insight into its physiological function and possible role in human disease.

PP01.154

Benzbromarone as a Novel Inhibitor of Melanoma Cells

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Abstract: Despite recent developments of immune- and targeted therapies, the overall survival for patients with metastatic melanoma still remains poor. Therefore, there is a need for additional effective melanoma therapies. We previously found that melanoma cells express much higher levels of EYA1 compared with benign melanocytes. EYA1 is a crucial signal transduction molecule involved in the regulation of DNA repair and apoptosis, and has been shown to promote tumorigenesis of breast cancer. In addition, research by others revealed that benzbromarone, a medication used for the treatment of hyperuricemia in several countries, has inhibitory activities against EYA1. Therefore, we hypothesize that benzbromarone can be used as a therapy for melanoma. To test this, we treated two melanoma cell lines (A375, which has V600E mutation of BFRAF gene that is responsive to vermurafenib therapy, and RPMI7951, which does not contain V600E mutation, and is non-responsive to vermurafenib treatment) with various concentrations of benzbromarone. It was found that benzbromarone can significantly inhibit the growth of these melanoma cells, with IC_{50} at 25.1 ×M and 30.3 ×M for these two cell lines, respectively. Further, addition of vermurafenib did not significantly alter the IC_{50} of benzbromarone for either cell line. We conclude that EYA1 inhibition via benzbromarone can efficiently inhibit the growth of melanoma cells independent of the V600E mutation status. Therefore, benzbromarone has potential to be

a novel melanoma therapy warranting further testing in animal models and eventually in human clinical trials.

PP01.155

Enriched Environment Reverses Hyperglycemia and Cognitive Impairmentin Stretozotocin Induced Diabetes

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Abstract: Diabetes is a heterogeneous disease resulted from genetic and environment factors. Environment can influence the physical activity accumulation and environment enrichment (EE) is a method to promote physical and cognitive activity for animals. However, there is a lack of information about the role of enrichment environment in rats. To study the effects of enrichment housing in diabetic animals, 21 wistar rats were distributed into three groups: Control animals kept in regular cages (C), Diabetic kept in regular cages (D) and Diabetic housed on enriched environment (DEE). Diabetes was induced by streptozotocin (50 mg/kg) intraperitoneal infusion. After eight weeks of experiment, we performed a cognition test (Open Field Test) in all three groups. Animals were then anesthetized and a bolus of insulin (2UI) was administered through hepatic portal vein. Three minutes after this administration, animals were euthanized to collect blood and soleus muscle. Blood biochemistry, and soleus proteins involved in the insulin signaling (AKT, pAKT, ROCK-2, RhoA, RhoE) were checked through colorimetric method and western blotting assay. Results: Cognitive disturbance (C: 38.447.0, D: 54.6413.6, DEE: 36.7420.0) and hyperglycemia were induced by strptozotocin and partially recovered by the enriched environment group (C: 92.3410.1; D: 410.3495.2, DEE: 305.2440.9; pM0.05). AKT phosphorylation, ROCK-2 levels, RhoA and Rhoe did not show any changes in all groups observed. In conclusion, maintenance in EE improved serum biochemistry, reducing glycaemia and triglycerides in diabetic rats.
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PP01.156

The Lysine Acetyltransferase NuA4 Regulates Glucose-Deprived Stress Granule Formation Through Cellular Acetyl-coA Levels

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Abstract: Eukaryotic cells form cytoplasmic RNA-protein aggregates or stress granules (SGs) under a variety of stress conditions and their formation is associated with both neurodegenerative diseases and cancer. For each stress condition distinct stress-activated signaling pathways regulate SG formation, however the molecular details of these pathways remain largely unknown. We have determined that the *Saccharomyces cerevisiae* lysine acetyltransferase complex NuA4 is required for SG formation specifically upon glucose deprivation but no other stresses tested. Similarly the Tip60 complex, the human homolog of the NuA4 complex, is also required for SG formation in human cell lines indicating that NuA4/Tip60 is a conserved signaling pathway regulating SG dynamics. Surprisingly we found that the impact of NuA4 on glucose-deprived SG formation is not through the regulation of core SG protein levels, or inhibition of translation, rather it is through regulation of acetyl-CoA levels. Cells in which the Acetyl-CoA synthetase *ACS1* is deleted, which have decreased acetyl-CoA levels, display increased SG formation upon glucose deprivation. In agreement, cells in which the Acetyl-CoA carboxylase *ACC1* is mutated or in cells exogenous treated with acetate, which have increased acetyl-CoA levels, glucose deprived SG formation is suppressed. Like NuA4, acetyl-CoA levels appear to only contribute to SG formation upon glucose deprivation and not other stresses. Remarkably we determined that mutants of NuA4 have increased acetyl-CoA levels, decreased Acc1 activity and mislocalized Acc1-GFP. Our observations indicate that NuA4 is regulating acetyl-CoA levels through Acc1 and that acetyl-CoA is acting as a signaling rheostat for SG formation upon glucose deprivation.

PP01.157

Role of eEF2K in DNA Damage Response Following Genotoxic Stress

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Abstract: Many of DNA damage inducing chemotherapeutic drugs preferentially kill cancer cells but they also have a negative impact on many of the normal cells in the body. Having a better understanding of how tumors are responding to the DNA damage caused by chemotherapeutic agents can improve the chemotherapy regimen and reduce the harm done on the patient. Eukaryotic elongation factor 2 kinase (eEF2K) is a regulator of mRNA translation which is over-expressed in medulloblastoma patients with worse prognosis. It was reported that eEF2K increases cellular sensitivity to inducers of DNA damage, including hydrogen peroxide and doxorubicin. We intend to define the mechanistic role of eEF2K in DNA damage response (DDR) and its role in sensitizing cells to genotoxic agents and ionizing radiation. To this aim, we treated wildtype (WT) and eEF2K knockout (KO) mouse embryonic fibroblasts with cisplatin and ionizing radiation. We found that eEF2K KO cells are more resistant to cisplatin treatment, as showed by using MTT assay and trypan blue assay. In addition, our data indicate that eEF2K KO cells exhibit defective induction of the prototypical ATM and ATR DDR pathways in response to cisplatin or ionizing radiation. This is correlated, in eEF2K KO cells, with a reduction in the recruitment of the DNA damage markers gH2AX and 53BP1 to the sites of damage under cisplatin treatment. Altogether, our works suggest that eEF2K is required for mediating the DDR in response to cisplatin and ionizing radiation, which may enhance cellular sensitivity to cisplatin.

PP01.158

The Role of MALT1 in Macrophages

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Abstract: MALT1 is a signalling molecule that acts as both a protein and a protease. MALT1 plays a key role in Nuclear Factor kappa B (NF-κB) activation downstream of the B and T cell receptor causing lymphocyte activation and proliferation. MALT1 also plays a role in macrophage activation by acting downstream of dectin-1, dectin-2, toll-like receptor 2/6 (TLR2/6), and TLR4 signalling pathways. A key role for MALT1 in intestinal inflammation was recently demonstrated because patients deficient in *MALT1* develop severe combined immunodeficiency accompanied by dramatic inflammation along the gastrointestinal tract. MALT1 contribution to inflammation has been attributed to B and T cells. However, the role of MALT1 in macrophage-mediated inflammation has not been investigated. Based on this, we hypothesize that MALT1 deficiency causes inflammation by increasing macrophage inflammatory responses. Our results show that MALT1 deficient murine macrophages have a lower inflammatory response than wild type macrophages. In contrast, inhibiting MALT1 activity increases inflammatory cytokine production by murine macrophages. We also found that stimulation of macrophages with TLR4 ligand increases both MALT1 expression and its activity in these cells. Taken together, our studies are consistent with a model in which MALT1 activity reduces pro-inflammatory macrophage responses but its scaffolding function increases macrophage inflammatory responses. In future studies, we will investigate the cell-specific contribution of MALT1 deficient macrophages to inflammatory disease by using mice with myeloid-specific MALT1 deficiency. These studies will provide critical information about the cell specific role of MALT1 and possible side effects of MALT1 inhibitors currently used for lymphoma treatment.

PP01.159

Molecular Organization and Interactions of the Fission Yeast Atg1 Complex

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Abstract: The budding yeast *Saccharomyces cerevisiae* Atg1 complex composed of Atg1, Atg13, Atg17, Atg29, and Atg31, represents a key model for understanding how the Atg1/ULK complex mediates initiation of starvation-induced autophagy. The human ULK complex consists of a novel subunit Atg101 of unknown function in place of Atg29 and Atg31. To probe the function of Atg101 and to gain insights into the molecular organization of the ULK complex, we have studied the fission yeast *Schizosaccharomyces pombe* Atg1 complex, which has a composition similar to the ULK complex (Atg1, Atg13, Atg17, and Atg101) but is more amenable to biochemical studies. A subunit interaction map generated based on pairwise pull down experiments between different *S. pombe* Atg1 complex subunits, and/or their core domains, revealed that Atg101 binds to the N-terminal HORMA domain of Atg13 but not to Atg17. Atg101 and Atg13 were recently shown to adopt HORMA domain structures, but our chemical cross-linking experiments show that these proteins do not self-associate. Using differential scanning fluorimetry, we found that Atg101 and Atg13 stabilize one another. *S. pombe* Atg17, according to our single-particle electron microscopy analysis, adopts an elongated rod-shaped dimeric architecture as opposed to the S-shaped conformation found for *S. cerevisiae* Atg17-Atg31-Atg29. *S. pombe* Atg17 could not rescue autophagy defects in a *S. cerevisiae* atg17D strain. Collectively, our findings show that Atg101 does not play the same role as the Atg29-Atg31 complex and suggest that *S. pombe* Atg17 might function in a different manner than its orthologue in *S. cerevisiae*.

PP01.160

Rapamycin Induces Changes in Genome Organization and Function Potentially Mediated by the STAT5A/B Transcription Factor

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Abstract: Rapamycin, commonly used as an immunosuppressant, has been proposed to extend health and lifespan via inhibition of the mammalian target of rapamycin (mTOR) pathway. Although the impact of rapamycin is extensively characterized in both cells and organisms at the molecular level, the impact of this compound on genome function (gene expression) and organization (folding of the genome within the nuclear volume) is poorly understood. We determined that treatment of healthy human fibroblasts with rapamycin resulted in a significant decrease in cell proliferation, changes in cell morphology and repositioning of chromosomes 10 and 18 within the nuclear volume. These observed changes were similar to those noted upon quiescence induction via serum deprivation. Regardless of these similarities, comparative transcriptome analyses of rapamycin-treated and quiescence-induced fibroblasts revealed divergent changes in gene expression. Rapamycin up-regulated cytokines in the interleukin-6 signaling cascade, whilst genes in the complement and coagulation cascade was upregulated in response to quiescence-induction. Genes up-regulated by rapamycin treatment demonstrated increased promoter occupancy of the transcription factor Signal Transducer and Activator of Transcription 5A/B. Our observations link mTOR inhibition via rapamycin with changes in genome function and organization and propose a mechanism that may play a role in regulating cellular health and longevity.

PP01.161

Peptides as Modulators of Cellular Responses to Neurotransmitters

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Abstract: The mechanism of cellular responses to small molecules consist of many different stages, in most cases the first of them is signaling from cell plasmatic membrane receptors. Pharmacological effects of many drugs is based on modulation of ligand - receptor binding functional activity. The main endogenous neurotransmitters of cellular excitation and inhibition processes are glutamate and GABA. In the work presented, we have studied the molecular base of action of short synthetic neuropeptides with double psychotropic effects (nootropic and anti-anxiety) - Selank (used in clinical practice) and its C-terminal fragment (RPGP). Were also researched molecular effects of potential neuroleptics - biologically active analogues of neurotensin: WPYF and APYF. By using the radioligand-receptor method of analysis of specific intermolecular interactions we found that neuropeptides above able to modulate [^{3}H]GABA, [^{3}H]Glu, [^{3}H]Ifenprodil, [^{3}H]Dopamine and some other tritium labeled neuromediators specific binding to rat brain cells plasmatic membranes. The joint action of specific ligands of some crucial neuroreceptor systems in the system of peptide + non-peptide allosteric modulator was investigated. We showed that peptides investigated and some of their synthetic derivatives able to modulate GABA, Glu and etc. specific binding within wide concentration range (from picoM to microM). Thus, the biological effects of some peptides were apparently due to a combination of the direct modulating action of the peptide on the target receptor, allosteric modulation of receptor activity and the initiation of the biochemical cellular mechanisms that determine the molecular basis of the physiological effect of the peptide.

PP01.162

The Role of the Mixed Lineage Leukemia Mutant MLL-PTD in Leukemogenesis

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Abstract: Acute myeloid leukemia (AML), the most common acute leukemia that affects adults, is a genetically heterogeneous disease with a poor prognosis. A mutation in the mixed lineage leukemia (MLL) gene that results in partial tandem duplication (PTD) of the N-terminal region is found in 5-10% of AML cases with normal cytogenetics. MLL-PTD has been shown to be a gain of function mutation and is associated with a worse prognosis compared to patients who do not harbour the mutation. The molecular mechanism through which MLL-PTD induces leukemic transformation is currently unknown. We hypothesize that the PTD mutation will cause deregulated binding to both co-factors and gene-loci as a result of its two additional DNA binding domains and increased size compared to wild type MLL (MLL-WT). In order to facilitate a comparative study between the wild type and mutant MLL, we have generated leukemic cell lines with inducible expression of FLAG-tagged MLL-WT or MLL-PTD and inducible downregulation of endogenous MLL using tetracycline-controlled transcriptional activation. Expression of the tagged MLL has been validated using immunofluorescence and qRT-PCR. In addition, we observed nearly a complete knockdown of endogenous MLL as seen by western blot. These cell lines will be tools for future genomic and proteomic analysis and will hopefully lead to the identification of MLL-PTD's role in leukemogenesis and provide new candidate targets for the design of antileukemic drugs.

PP01.163

Set2-Mediated H3K36 Methylation Signals for the Selective Suppression of Intragenic Antisense Transcription

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Abstract: Maintenance of a regular chromatin structure over the coding regions of genes occurs co-transcriptionally via the 'chromatin resetting' pathway. This feature ensures the assembly of a stable chromatin structure, which suppresses spurious transcription initiation. One of the central players in the co-transcriptional 'chromatin resetting' pathway is the histone methyltransferase Set2. Here we show that the loss of Set2 in yeast, *Saccharomyces cerevisiae*, results in unfettered transcription initiation of antisense transcription units that are usually embedded within body of protein coding genes. These RNA are distinct from the previously identified non-coding RNAs and cover 11% of the yeast genome. We demonstrate that the co-transcriptional addition of the H3K36 methyl mark by Set2 over antisense RNA start sites is responsible for their suppression. Therefore, these RNA species have been named Set2-repressed antisense transcripts or SRATs. Despite being an elongation factor, Set2 loss does not affect the abundance of the sense transcripts. The strand-independent addition of H3K36 methylation, instituting strand-specific differences in transcriptional outcomes, highlights a key regulatory feature of interleaved transcriptomes. Finally, we tease out the multiple molecular mechanisms by which Set2-mediated H3K36 methylation signals the suppression of intergenic antisense transcription.

PP01.164

Differential Immune Responses Between Two Salmon Species to Infestation With Caligus Rogercresseyi

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Abstract: *Caligus rogercresseyi* is the most problematic parasite of salmonids in seawater in Chile. The molecular mechanisms implicated in resistance/susceptibility among salmon species are scarcely understood. The aim of this study was to investigate the transcriptional responses of immune markers in the muscle/skin of susceptible and resistant salmonids to *C. rogercresseyi* infestation. The mRNA expression levels of IL-1β, IL-6, IL-10, IL-12 and TNFα in Atlantic and Coho Salmons were studied throughout the infestation period from copepodids (1 dpi) at chalimus (11 dpi) and then to adult lice (19 dpi). A differential immune response for both salmon species was early detected for TNFα (1 and 11 dpi). Also, at 11 dpi, Chalimus step, slight differences were detected for IL-12. The most marked differences were observed for adult caligus step (19 dpi) for IL-1β, IL-6 and IL-10. Interestingly, differential immune responses were also detected between healthy and damaged muscle/skin for both species. We established the first evidence for temporal expression of early defense and inflammatory mediators both at the site of fish-caligus interaction and at non-interaction sites in the skin of resistant and susceptible salmonids. In addition, we differentiate between the responses to infection and mechanical trauma. Impaired genetic expression in these pathways may be evidence for species specific pathways of susceptibility to the parasite between Atlantic and Coho Salmons. The knowledge about the mechanism involved in resistance/susceptibility observed between salmonids will allow us to develop effective treatments against this parasite. Funding: InnovaChile (CORFO) 14IDL2-30112. Fondecyt 1150934 and Fondap 15110027 from CONICYT, CHILE.

PP01.165

Pre-Clinical Study to Assess Signalling Pathways That Drive Recurrence of HER2+ Breast Cancer

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Abstract: The objective of our project was to identify key players that drive recurrence of HER2+ breast cancer. Previously we discovered two drugs that when combined, inhibited the growth of HER2+ breast cancer. Specifically, the combination of AZD8055 and 2-DG significantly reduced mammary gland tumourigenesis by inhibiting mTOR activity, and cancer cell metabolism[1]. Because the standard treatment of care for HER2+ breast cancer is Trastuzumab, we were interested in whether combining Herceptin® with inhibitors of mTOR and cancer metabolism, would be effective at inhibiting tumour growth and recurrence. The outcome of our pre-clinical study confirms the use of combination therapy to prevent recurrence of HER2+ breast cancer using a novel drug combination administered simultaneously with Trastuzumab. Our study identifies markers of metabolism that contribute to recurrence of HER2+ breast cancer. These new markers will allow for the design of new therapies that would improve clinical outcomes by blocking HER2, pro-survival pathways and energy metabolism. The results from our study will lead the way for a Phase I clinical trial designed to target proteins that are involved in driving HER2+ breast cancer recurrence, thereby curing HER2+ breast cancer. *This work was funded by the Dalhousie Medical Research Foundation, the Beatrice Hunter Cancer Research Institute and the Canadian Breast Cancer Foundation.* 1. Andrade-Vieira, Goguen, Bentley, Bowen & Marignani *Oncotarget* **5**, 12738-12752 (2014).

PP01.166

Interactome Studies Reveal Critical Roles of Carbonic Anhydrase IX (CAIX) in Tumour Progression Pathways

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Abstract: In solid tumours, the tumour microvasculature is often disorganized leading to hypoxic tumour microenvironments. Carbonic Anhydrase IX (CAIX) is a hypoxia induced enzyme that enables hypoxic tumour cells to maintain an optimum intracellular pH. CAIX is a transmembrane protein that has been shown to contribute towards several processes critical for cancer progression such as cell survival, adhesion, migration, invasion and the acquisition of chemo- and radio-resistance. We have previously shown that CAIX is a marker of poor prognosis of breast cancer specifically the triple negative breast cancer. Using a unique proximity dependent biotinylation techniquecalled BioID, we have now identified the protein interactome of CAIX that has revealed interactions of CAIX with the collagen degrading enzyme, Matrix metalloproteinase-14 (MMP14), critical cell adhesion proteins such as Integrins - α2β1, α3β1, α6β1 and transport proteins such as the bicarbonate transporter SLC4A7 and the amino acid transporter- CD98hc. The validation of these interactions by co-immunoprecipitation and immunofluorescence studies reveals that CAIX co-localizes with these proteins at the leading fronts and invasive cellular structures such as the pseudopodia and invadopodia of migrating cells. We show that the collagen degrading activity of purified MMP14 increases with CAIX concentration. However, upon the arrest of proton production using deuterium-di-oxide, CAIX does not have an effect on MMP14 activity. We demonstrate that CAIX plays a critical role in modulating the matrix remodelling activity of MMP14 through proton production at the invasive structures of hypoxic, migrating tumour cells.

PP01.167

An Affinity Protein Crystallographic Approach to Identifying High Affinity Cathepsin K Inhibitors from Natural Extracts.

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Abstract: Cathepsin K is the predominant lysosomal cysteine protease involved in bone resorption. It is therefore a major target in osteoporosis and bone cancer treatment. Several cathepsin inhibitors have been isolated from natural sources including E-64 and leupeptin. Isolation of novel pharmacologically active compounds from natural products can however be laborious and unpredictable. The costs and timelines associated with this process has been a major bottleneck in pharma drug discovery programs. Affinity protein crystallography is a new method that significantly shortens the timeline from natural product crude extract to structure elucidation. Here we use affinity protein crystallography to selectively pull out lichostatinal, a cathepsin K inhibitor from semi-pure fractions of an actinomycetes culture extract. We compare this technique to the traditional assay guided purification approach and show that affinity protein crystallography isolated the most affine but otherwise difficult to purify compound in the semi-pure extract. Lichostatinal was further verified by synthesis and kinetic characterization.

PP01.168

Molecular Architecture of the Yeast Elongator Complex Reveals an Unexpected Asymmetric Subunit Arrangement

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Abstract: Elongator is a ~850kDa protein complex that is involved in multiple processes from transcription to tRNA modification. It is conserved from yeast to humans and composed of two copies of six subunits (Elp1-Elp6). Despite the wealth of subunit structural data, the molecular basis of how Elongator exerts multiple activities remains unclear. We have characterized the structure of the full yeast Elongator by single-particle electron microscopy (EM). Our analysis revealed that the heterodimeric Elongator adopts a "moth" shaped overall architecture and an asymmetric subunit arrangement resulting from the hexameric Elp456 subassembly anchored to one of the two lobes. A molecular model of Elongator constructed using the EM data combined with crosslinking coupled to mass spectrometry suggested that Elongator contains two catalytic Elp3 subunits in two distinct environments: one positioned adjacent to the Elp456 ATPase subassembly and one in a more solvent accessible location.

PP01.169

21-900, a Novel Dual-Targeting HDACs/Microtubule Inhibitor, Inhibits Human Leukemia Cells Growth in Vitro and in Vivo

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Abstract: Malignant tumor has been the major cause of death in the world. When tumors are difficult to be removed simply by surgery, chemotherapies and targeted therapies become important treatments. The purpose of this study is to evaluate the anti-tumor effects of a novel synthetic HDAC and tubulin dual inhibitor, 21-900, in human leukemia cells, and to further study the mechanism of action in vitro and in vivo. We first determined a series of 1-benzyl indoles as potent anti-cancer drugs which are more cytotoxic to human leukemia HL-60 cells. Among these compounds, 21-900 significant exhibits a cytotoxic effect in human leukemia cancer cell HL-60 and MOLT-4. 21-900 also exhibited potent HDAC inhibition with an IC50 value of 610 nM. We further demonstrated that 21-900 induced cell cycle arrest at G2/M phase in HL-60 and MOLT-4 cell line, with the increase of sub G1 population. Then tubulin polymerization assay and immunofluorescence stain suggested that 21-900 was similar to vincristine in causing microtubule depolymerization. 21-900 could affect microtubule dynamic accompanied by the up-regulation of mitotic marker, MPM2. Furthermore, 21-900 could influence the Bcl-2 family proteins and induce cell apoptosis in a concentration-dependent manner. *In vivo* animal model experiments demonstrated that 21-900 significantly inhibit HL-60 and MOLT-4 xenograft tumor growth without bodyweight loss, indicating 21-900 has tumor suppressive effect and low toxicity. In summary, 21-900 possesses strong in vitro and in vivo activity against human leukemia cells, representing a potential therapeutic approach for cancer therapy.

PP01.170

Antiaging Effect of Galactomannan Fraction From Arenga Pinnata in Vitro

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Abstract: Cosmeceuticals refer to natural cosmetics with medical-like benefits due to their bioactive contents. Sugar palm fruit (*Arenga pinnata*) extract has been claimed for its anti-aging

effect *in vitro*. However, its active compounds for cosmeceuticals is still unclear. This study was aimed to extract galactomannan from *A. pinnata* and test its efficacy for tyrosinase inhibition, antioxidant, and antiphotoaging activities *in vitro*. Galactomannan from *A. pinnata* was extracted by freeze drying and identified its chemical compounds by using pyrolysis-Gas Chromatography Mass Spectrometry (py-GC/MS). Galactomannan was tested for its tyrosinase inhibition in both cell-based (melanocytes) and enzymatic assays, antioxidant activity using ferrous chelating activity/FCA assay, and antiphotoaging activity for inhibiting the gene expression of matrix metalloproteinase-1/MMP-1 and MMP-13 in macrophages using Quantitative Real-Time Polymerase Chain Reaction/qRT-PCR analysis. Identification of galactomannan compound from *A. pinnata* by py-GC/MS mainly consisted of oxonium ion and glucoside. For cellular assay, galactomannan at 5 ×g mL^{-1} inhibited >50% of tyrosinase activity; while at the enzymatic level, galactomannan at similar concentration showed less tyrosinase activity inhibition (~20%). FCA results showed that galactomannan at 10 ×g mL^{-1} exerted >50% of antioxidant activity. The qRT-PCR data indicated that galactomannan at 5 ×g mL^{-1} inhibited >50% of MMP-1 and MMP-13 gene expressions in macrophages. Galactomannan fraction from *A. pinnata* has efficacy for enlightening effect, antioxidant, and anti-photoaging activity in dose-independent pattern, indicating its cosmeceutical effects for skin healthcare.

PP01.171

Hypocholesterolemic Effect of Orally Curcuminoid Fraction in High-Cholesterol-Fed Rats

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Abstract: Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum levels of low density lipoprotein (LDL-C) and blood cholesterol. Alteration in cholesterol and triglycerides metabolism as a result of hypercholesterolemia has been shown to affect oxidative stress biomarkers and promote production of reactive oxygen species (ROS). The present work was aimed to study the efficacy of curcuminoid fraction from *Curcuma xanthorrhiza* in reducing blood cholesterol level and stress oxidative-related genes including cluster of differentiation (CD44), intercellular adhesion molecule 1 (I-CAM), Inducible nitric oxide synthase (iNOS), and lectin-like oxidized low-density lipoprotein (LDL) receptor-1 (LOX-1) in high-cholesterol-fed rats *in vivo*. Twenty male Sprague-Dawley rats were divided into four groups, namely normal group diet, high cholesterol diet (HCD) 2%, HCD + 100 mg/kg bw curcuminoid fraction, and HCD + 300 mg/kg bw curcuminoid fraction for 4 weeks. Lipid profiles were measured at day-1, -14, and -28. Vascular tissues and organs from lung and liver were collected for RNA extraction, followed by quantitative analysis using Real-Time PCR. Among lipid parameters, total cholesterol levels were significantly reduced after treatment with curcuminoid fractions (100 and 300 mg/kg bw) compared to that of HCD group. Real-Time PCR results showed that curcuminoid fractions at 100 and 300 mg/kg bw significantly inhibited the gene expression of CD44, ICAM-1, iNOS, and LOX-1 from all tissues, indicating its potential hypocholesterolemic effect via mediating stress oxidative-related genes in rat model *in vivo*. In summary, oral administration of curcuminoid fraction from *C. xanthorrhiza* may be applied for prevention of hypercholesterolemia-induced atherosclerosis *in vivo*.

PP01.172

Role of STAT3 in IL-6 Mediated Drug Resistance in Human Medulloblastoma

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Abstract: Chemotherapy is one of the principal modes of treatment for cancer, but the effectiveness of chemotherapy is limited by drug resistance. In this study, we have evaluated the role of STAT3 in a tumor microenvironment mediated drug resistance in human medulloblastoma. We have established that STAT3 is not constitutively activated in Meb-Med8A human tumor cell line but can be rapidly activated by tyrosine phosphorylation when treated with Interleukin-6 (IL-6) alone or in combination with IL-6 and soluble IL-6 receptor. Several stable chemoresistant variants of parental Meb-Med8A-S were made by gradual drug (vincristine) selection. The drug resistant variants, Meb-Med8A-R exhibited higher STAT3 activation upon treatment with IL-6 in a dose-dependent manner. We have also demonstrated a biphasic tyrosine phosphorylated activation of STAT3 when cells are exposed to IL-6 for a short period of time. Paracrine and autocrine signalling can release IL-6 in the tumor microenvironment and induce an expression in adjacent cells. Conditioned media from IL-6 stimulated cells invoked a stronger STAT3 activation even after 48 hours of initial exposure indicating that initial 30 minutes' exposure of cells to IL-6 is necessary and sufficient for a prolonged STAT3 activation. Abnormal constitutive activation of STAT3 has been associated with drug resistance and poor prognosis of the disease. The IL-6/STAT3 is an interdependent pathway that contributes to drug resistance between the medulloblastoma cells and their tumor microenvironment.

PP01.173

The Unexpected Importance of Nucleoporin Nup153 in the Intracellular Traffic of Influenza A Virus

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Abstract: Influenza A virus (IAV) continues to be a worldwide major threat to human health. A good strategy for the development of new influenza antiviral drugs is to interfere with the intracellular traffic of its viral genome. A genome-wide RNAi screen identified the nuclear pore complex protein Nup153 as a host factor required for IAV infection (König et al. 2010, *Nature* 463:813-817). We investigated the role of Nup153 during IAV infection by infecting HeLa cells where Nup153 was knocked down (KD) with siRNA.

We found that these cells produced less infectious particles than control cells, however, no significant changes were detected in the nuclear import of the influenza nucleoprotein (NP) or the nuclear import of chimera proteins containing NP's nuclear localization signals. To explain why the Nup153 KD cells produced less infectious viral particles, we next analysed the sub-cellular localization of NP, M1, and HA at different times of infection in the Nup153 KD cells, and found a deficiency in cytoplasmic trafficking of viral components in these cells. This is the first time that intracellular traffic changes of this nature have been observed in relation to Nup153. Our results indicate that there is defective assembly or budding of progeny viral particles in the Nup153 KD cells. We also demonstrated that these defects could be explained because Nup153 RNAi depletion results in a plethora of cellular defects, including defects in endocytic organelles and the cytoskeleton. Understanding the function of Nup153 will help to unveil important insights of the IAV infectious cycle.

PP01.174

Role of Vimentin During the Early Stages of Influenza A Virus Infection

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Abstract: Influenza A virus exploits the subcellular transport machinery during the early stages of infection. Actin filaments and microtubule facilitate the trafficking of virus-containing endosomes towards the perinuclear region; however, the role of vimentin remains to be determined. To study the role of vimentin during influenza A virus infection, we infected vimentin-null ($vim^{-/-}$) mouse embryonic fibroblasts (MEFs) with influenza A virus and found that the lack of vimentin significantly reduced viral RNA and viral protein expression and production of viral progeny. These results indicate that vimentin is essential for influenza A virus infection. As influenza A virus uses the endocytic pathway during the early stages of infection and endosomal organelles are know to be mislocalized in vimentin-null cells, we investigated the kinetics of the co-localization of the virus with several organelles of the endocytic pathway during infection of both $vim^{-/-}$ MEFs and HeLa cells where vimentin was knocked down (KD) with siRNA. Our results showed that endosomal trafficking was compromised in both $vim^{-/-}$ MEFs and vimentin KD cells and that the lack of vimentin led to accumulation of incoming influenza A virus within late endosomes. Using a pH-sensitive fluorescence marker (pHrodo-EGF) we further confirmed that acidification of endocytic organelles is significantly impaired in vimentin-null cells, which explains the retention of incoming virions in late endosomes. These findings are the first to demonstrate that vimentin is critical for influenza viral infection as it facilitates endosomal trafficking and acidification, and mediates viral genome penetration into the cytoplasm to propagate the infection.

PP01.175

RbmA: A Multifunctional Protein Component of the Vibrio Cholerae Biofilm Matrix

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Abstract: Vibrio cholerae, the causative agent of cholera, is a gram negative bacterium, which may swim freely, or grow in sessile biofilms associated with abiotic surfaces, zoo-plankton, mollusks, or crustaceans in estuarine and brackish waters. Vibrio cholerae biofilms are involved in many aspects of the pathogen's life-cycle, constituting a possible source of antibiotic resistances, and being very important for intestinal colonization. Particularly during exopolysaccharide-dependent biofilm formation, secreted proteins of the rbm gene cluster, including RbmA, RbmC, and Bap1 are key to biofilm ultrastructure, along with the vibrio polysaccharide (VPS) itself. While RbmC and Bap1 have been linked to biofilm-surface interactions , RbmA surrounds cells within the biofilm, which results in improved mechanical strength, and better biofilm accumulation by a not very well understood mechanism, but possibly involving glycan binding. Furthermore, RbmA has been linked to micro-colony formation during V. cholerae pathogenesis, significantly enhancing the infectivity of the pathogen. Here, we elucidated the RbmA crystal structure, as well as demonstrating glycan binding activity. Furthermore, we observed two distinct proteolytic pathways for RbmA. In the first one, protease mediated post-translational modification yielded a different RbmA isoform, RbmA*, involved in VPS independent cell recruitment. The second pathway, which is phosphate and magnesium dependent, possibly leads to RbmA inactivation, and may be involved in biofilm dispersal.

PP01.176

Familial Hypercholesterolemia Registry: Lifesaving Potential

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Abstract: Cardiovascular disease (CVD) is now the leading cause of death worldwide. Atherosclerosis is the most common pathological change underlying CVD, with high cholesterol levels proven as a major risk factor for its development. Familial hypercholesterolemia (FH), a common autosomal dominant genetic disease, causes high cholesterol levels in the bloodstream, leading to premature CVD. With a prevalence of 1:500 in the population, approximately 20 million people worldwide and around 8000 in British Columbia are carriers of one of the genes for FH. Although more common than cystic fibrosis, vast majority of FH cases still remain undiagnosed, which implies that health professionals lack awareness of FH, its diagnostic features, and consequences. The purpose of this project is to create a registry with the diagnosis of FH in British Columbia to simplify education and treatment, ultimately reducing morbidity and mortality from CVD through early diagnosis and effective disease management. Patients with FH were identified based on their family history, physical examination, and laboratory results and stratified as definite, probable, or possible FH according to

their final score on The Dutch Lipid Clinic Network FH critera. Cascade screening was used to to track the mode of inheritance of the disease to identify affected family members. Thus far, we have identified over 1000 individuals with FH based on chart review, with 690 consented to join the registry. Further research will aim to identify and educate FH individuals, their families, and their physicians regarding the appropriate management of hypercholesterolemia using lifestyle measures and medications.

PP01.177

Comparison of the Anti-Resorptive Activities of an Ectosteric With an Active Site Inhibitor of Cathepsin K

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Abstract: Cathepsins play a vital role in numerous physiological processes such as bone remodeling, cardiovascular disorders, cancer, lung disease, immune defects, and wound healing. Cathepsin K (catK), a cysteine protease, is of particular interest as this enzyme is critically involved in bone degradation and at the same time responsible for other complications. The currently available active site inhibitors of CatK interfere with osteoclast collagenolysis; however, they also inhibit the activity of CatK toward other proteins. Therefore, it is important to find alternative ways to inhibit bone resorption that avoid off-site and off-target effects. The present study investigates the anti-resorptive effect of an exosite inhibitor that selectively inhibits only the therapeutically relevant collagenase activity of CatK without interfering with other pathways. Human osteoclasts and fibroblasts were used to analyze the effect of the exosite inhibitor, ortho-dihydrotanshinone (DHT1), and the active site inhibitor, odanacatib (ODN), on bone resorption and TGF-ß1 degradation. DHT1 and ODN selectively inhibit the collagenase activity of CatK without affecting the viability of osteoclasts with IC_{50} values of 60.140.4 nM and 14.244.4 nM, respectively. However, DHT1 did not affect the turnover of fibrosis-associated TGF-ß1 in fibroblasts, whereas 500 nM ODN was inhibitory. In conclusion, exosite inhibitors have the intrinsic advantage of not blocking the active site of CatK and thus not interfering with physiologically relevant non-collagen substrates of this protease such as TGF-ß1, which in part may account for some of the side effects seen.

PP01.178

Comparing the Mechanisms of Ectosteric and Allosteric Inhibitors of Cathepsin K

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Abstract: Enzyme activity attenuation can occur through active site inhibition or by exosite regulation away from the active site. One type of exosite regulation is allosteric regulation, which is defined as a conformational change in the active site mediated by secondary binding sites. Ectosteric sites are exosites which do not elicit conformational changes in the enzyme and can interfere with protein-substrate or protein-protein interactions. Cathepsin K (CatK) is a cysteine protease responsible for the degradation of collagen and elastin in bone and blood vessels. CatK has been shown to contain ectosteric and potentially allosteric sites. Recent studies demonstrated that dihydrotanshinone (DHT1) specifically inhibits the collagenase and elastase activities of CatK by targeting these ectosteric sites whereas an allosteric inhibitor, NSC13345, leads to a hyperbolic-type inhibition of several substrates. Using crystallography, molecular docking, site directed mutagenesis and enzymatic assays, we investigated the inhibitory mechanism of both compounds in more detail. Here, we demonstrate that NSC13345 binds to CatK in a non-productive manner at various non-active sites sites but only its binding in the vicinity of the active site causes an hyperbolic-type inhibition of the hydrolysis of certain substrates. This suggests that NSC13345 behaves as a different type of ectosteric inhibitor rather than an allosteric inhibitor. In contrast, the ectosteric inhibitor, DHT1, exhibits high potency and selectivity for the inhibition of collagen and elastin degradation without disrupting active site activity whereas high inhibitor concentrations also partially block the active site resulting again in a hyperbolic-type inhibition of the degradation of several non-matrix substrates.

PP01.179

Target Protein-Protein Interactions of p53 Through Segmental Isotopic Labeling and NMR

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Abstract: The p53 protein is an important tumor suppressor that contains multiple domains. The N-terminal transactivation domains TAD1 and TAD2 of p53 are responsible for specific protein-protein interactions with diverse families of proteins that activate transcription of stress response genes and direct activate apoptotic machinery at the mitochondria. The isolated transactivation domains of p53 have been extensively studied in literature but so far there's little structure information available in context of the full-length protein. Here, we applied divide and conquer approach to selectively label the first 93 amino acids of p53 (termed N-tail) with ^{15}N and/or ^{13}C through segmental isotope labeling. We are aiming to obtain structural information of the naked N-tail in p53. Moreover, we aim to solve the structure of N-tail when bound to

anti-apoptotic Bcl-xL protein. Together, the structural information will provide us with deeper insight into p53 function and may open up new avenue for treatment of cancer and other p53-related diseases.

PP01.180

Effect of Senescent Cell Secretome on the Inflammatory Response Orchestrated by Macrophages

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Abstract: Introduction: Cellular senescence is an essential tumor suppressor mechanism preventing the proliferation of damaged cells. However, senescent cells display a senescence-associated secretory phenotype (SASP) containing pro-inflammatory factors with potentially opposing effects for the organism. For example, SASP is beneficial when promoting tissue repair, but is detrimental when contributing to age-associated organ dysfunctions. Thus, understanding why and how senescent cells accumulate in tissues is key. The actors leading senescent cell clearance are still ill-defined but immune cells like macrophages (Mϕ) seem to be involved. We hypothesize that senescent cells, via SASP, modify Mϕ inflammatory phenotype helping them orchestrate senescent cell clearance. **Methods and Results:** FACS and multiplex protein secretion data showed that human Mϕ can develop a SASP-specific inflammatory profile characterized by pro-inflammatory cytokines (IL-6, IL-1b) and anti-inflammatory surface markers (CD206). Human SASP also impacts monocytes and macrophages behaviors as assessed by migration and invasion assays. High-content quantitative imaging of co-cultures assays showed that activated NK cells specifically kill senescent cells and that CD8+ T cells are essential for this process. Mϕ can't directly kill senescent cells but they restrain NK-mediated killing. **Conclusion:** In light of our data, Mϕ play a limited role in the direct elimination of senescent cells but orchestrate the activity of other immune cells involved in this process. A better understanding of senescent cell clearance could help suggest therapies centered around the manipulation of Mϕ responses to SASP in order to promote an ordered resolution of tissue damage in diseases associated with aging and leading to inflammation.

PP01.181

Signaling Pathways Regulating Heme Biosynthesis in the Drosophila Ring Gland

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Abstract: Steroid hormones play fundamental roles in development and disease. In insects, the molting hormone ecdysone is produced from dietary cholesterol in the prothoracic gland (PG). In *Drosophila*, PG is part of the ring gland that comprises two other glands. Notably, most ecdysone-producing enzymes are cytochrome P450 proteins, which require heme as a cofactor. However, little is known about the mechanisms by which heme synthesis is regulated to sustain ecdysone production in the ring gland. To identify genes essential for heme biosynthesis and its regulation in ring gland cells, we carried out a genome-wide RNAi screen to identify lines that show heme-deficient phenotypes. Heme deficiency usually results in an accumulation of its precursors known as protoporphyrins, some of which fluoresce under UV light. Strikingly, among the 34 hits that cause this phenotype, we have identified transcription factors, signaling molecules, and mitochondrial components, most of which have no unknown link to heme. Here, we present data on *Spätzle5*, a *Drosophila* gene with homology to human neurotrophins. *Spätzle5* has a novel role in controlling ecdysone synthesis via modulating heme synthesis in PG. It is also required for nitric oxide (NO) production possibly through controlling the activity of nitric oxide synthase (NOS). PG-specific NOS-RNAi results in similar phenotypes as *Spätzle5*-RNAi, consistent with the idea that Spätzle5 controls NO signaling to govern heme production, which in turn controls ecdysone output. These data shed new light on how the regulation of heme synthesis is tied to steroid hormone production, with potential implications for human disease models.

PP01.182

Polygonum Bistorta Exerts Anti-Cancer Effects Through Perturbation of Adhesion Dynamics and ROS-Induced Aggresome Formation

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Abstract: Polygonum bistorta is a widely used herb in traditional Chinese medicine for heat clearing and anticancer activities, although the molecular mechanisms for its anti-cancer properties remain unclear. The aim of this study was to evaluate the antitumor effects of P. bistorta (PB) water extract in vitro and in vivo in human hepatocellular carcinoma Hep3B and HepG2 cells. Results showed that PB (60–240 ×g/mL) caused cell death by decreasing cyclin and cyclin-dependent kinase (CDK) expression and inducing cell autophagy and apoptosis in a dose- and time-dependent manner. In addition, LC3 punctuation, caspase activation, and PARP cleavage were induced. Nevertheless, PB restricted cell motility by deregulating cytoskeleton distribution in Hep3B cells which may in part caused by polyphenols gallic acid, 3,4-dihydroxybenzoic acid, and chlorogenic acid. Furthermore, PB-induced reactive oxygen species triggered the formation of ubiquitinated protein aggregates that may be involved in suppression of cancer cell motility and survival. Oral administration of PB delayed Hep3B and HepG2 tumor growth in a xenograft model without adverse effects. This study provides evidence that PB may represent a safe and potent alternative therapy for patients with hepatocellular carcinoma.

PP01.183

Deciphering Alzheimer's Disease Pathology Using Clinical Proteomic Tools

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Abstract: Protein interaction networks play a vital role in sophisticated cell signalling. Deciphering the identity and dynamics of an interacting protein in a complex network is important for gaining insight into many aspects of Alzheimer's disease (AD). A number of studies implicates the potential involvement of GAPDH in neurodegeneration, a major glycolytic enzyme with multiple non-glycolytic functions, diverse cellular localizations and numerous interaction partners. In this study we employed Blue native PAGE to elucidate the interacting partners of GAPDH in human cortex of ageing AD brain. The present study aims to investigate the functional interaction of brain proteins and the altered expression of interacting components in a complex. Thirteen protein complexes from autopsied human brain cortical tissue were isolated and separated on BNPAGE gel into five to eight components. Using Orbitrap MS analysis ,we report here the discovery of novel binding partners of GAPDH in AD brain, including actin cytoplasmic, microtubule associated protein 1B, glial fibrillary acidic protein, myelin proteolipid protein, acyl amino acid releasing enzyme and cytochrome c oxidase subunit 2. Protein expression analysis reveals differential expression of proteins among control and AD subjects. A cardinal interaction present between GAPDH and actin was confirmed in our study by co-immunoprecipitation not reported before in AD. The canonical pathways and possible networks of the identified proteins were determined by Ingenuity Pathways Analysis (IPA) software. Our study adds new insight in AD research by exhibiting the involvement of GAPDH in cytoskeletal architecture, which may influence the pathogenesis and progression of this neurodegenerative disease.

PP01.184

Microbial Community Structure Determined by Effector-Immunity Modules

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Abstract: Gram-negative bacteria employ a large number of antimicrobial effectors to modulate the neighbouring microbial community. Effectors intoxicate susceptible species but spare own species that possess cognate immunity proteins. The diverse effector-immunity protein modules likely play a critical role in the warfare of microbes. Using the type 6 protein secretion system as a model, our study demonstrates that effectors possessing different toxicities are key determinants of the relative fitness between two antagonistic competing species. In contrast with immunity protein-dependent intraspecies protection, we show that effectors confer interspecies protection and propose a model for establishment of distinct community boundaries between competing species. This simplified model provides theoretical insights toward understanding the complex interactions and dynamic changes within microbial communities.

PP01.185

Adaptors Recruit the Yeast Chorein Homolog Vps13 to Distinct Membranes in the Cell

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Abstract: Chorea-acanthocytosis and Cohen syndrome are rare, neurological disorders that arise from loss-of-function mutations in two members of the VPS13 family, VPS13A/Chorein and VPS13B. Chorea-acanthocytosis causes involuntary jerking movements (similar to Huntington's disease) and abnormally-shaped red blood cells while features of Cohen syndrome include developmental delay, weak muscle tone, and small head size. Yeast has a single Vps13 protein that shares conserved domains with both VPS13A/Chorein and VPS13B. Recent work has suggested that the spatio-temporal localization of yeast Vps13 to different organelle membranes is largely controlled by adaptor proteins. For example, Spo71, a meiosis-specific adaptor, recruits Vps13 to the prospore membrane where it regulates phosphoinositide 4-phosphate levels. During mitotic growth, Vps13 plays a role in Golgi to vacuole trafficking, however the mechanism of this function is still unknown. We identified a new Vps13 adaptor that can localize Vps13 during mitotic growth. Additionally, we identified a conserved Vps13 region, the domain of unknown function (DUF) 1162, as the adaptor-binding domain. We showed that this domain is sufficient for binding the two known Vps13 adaptors and has a set of 6 conserved repeats that are important for the trafficking role of Vps13. This study gives novel insights into Vps13 localization and function. Furthermore, it suggests new avenues of research for understanding how VPS13A and B can localize to different membranes in human cells and how each *VPS13* gene is linked to a unique disease.

PP01.186

Multiple Roles of Z,Z-Farnesyl Diphosphate Synthase From the Wild Tomato Solanum Habrochaites

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Abstract: *Z,Z*-farnesyl diphosphate synthase (zFPS) is a short-chain prenyltransferase (PTS) from wild tomato (*Solanum habrochaites*). It displays multiple functions to synthesize prenyl diphosphates of various skeletal structures. The major product, *Z,Z*-farnesyl diphosphate (*Z,Z*-FPP), serves as a substrate for class

IIsesquiterpene synthesis that produces diverse phytochemicals in glandular trichomes of tomato. To elucidate the catalytic mechanism, the crystal structure of zFPS was determined. Among the many catalytic residues identified, H103 was shown to affect the enzyme's product distribution. Novel products including lavandulyl diphosphate (LDPP) and an unidentified compound synthesized by irregular head-to-middle condensation mechanism were observed. Monoterpenes products including limonene and α-terpineol revealed from mutagenesis assays also showed the potential cyclase functions of zFPS. Moreover, from the enzyme-substrate complex structures, the binding modes of Mg^{2+} ion as a cofactor and the key role played by the C-terminus in catalysis were both elucidated, providing new insights into the short-chain *Z*-type PTS.

PP01.187

PTP1B Oxidation Promotes a Novel Interaction With 14-3-3z

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Abstract: Despite the contribution of cardiac hypertrophy to heart failure, molecular mechanisms involved in this transition remain unclear. However, work from several groups point towards a critical role for reactive oxygen species (ROS) in this process. Importantly, the inactivation of the catalytic cysteine of Protein Tyrosine Phosphatases (PTPs) by ROS inhibits their ability for phosphotyrosyl hydrolysis, perturbs the delicate balance between the actions of PTPs and protein kinases, and potentially integrates ROS signalling to control signal transduction in cardiac hypertrophy and heart failure. We explored whether PTPs were inactivated in hypertrophying hearts and we identified PTP1B as a target of ROS in these conditions. We modeled the tridimensional structure of the reversibly oxidized form of PTP1B and synthetized a peptide that is exposed to the cytosol in these conditions. We used this peptide to pull-down, purify and identify new interacting partners. Using this approach, we identified 14-3-3z by mass spectrometry as a major interacting partner. We then induced PTP1B oxidation in cells using EGF, and confirmed that 14-3-3z interacted with PTP1B in a transient manner. As expected, this interaction was prevented by treating cells with N-acetylcysteine. Moreover, a stable interaction was detected between 14-3-3z and PTP1B (C215A, S216A), a mutant known to adopt the oxidized conformation of the enzyme. Finally, GST× was also identified as a potential partner of the protein complex. We are currently exploring whether a protein complex formed of 14-3-3z and GST× participate in the regulated reactivation of PTP1B and how this affects cardiac hypertrophy.

PP01.188

Activation of Aurora a Kinase Sustains the Stem Cell Characteristics of Glioblastoma Cells

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Abstract: Fibroblast growth factor 1 (FGF1) binds and activates FGF receptors, thereby regulating cell proliferation and neurogenesis. Human FGF1 gene 1B promoter (-540 to +31)-driven SV40 T antigen has been shown to result in tumorigenesis in the brains of transgenic mice. FGF1B promoter (-540 to +31)-driven green fluorescent protein (F1BGFP) has also been used in isolating neural stem cells (NSCs) with self-renewal and multipotency from developing and adult mouse brains. In this study, we provide six lines of evidence to demonstrate that FGF1/FGFR signaling is implicated in the expression of Aurora A (AurA) and the activation of its kinase domain (Thr288 phosphorylation) in the maintenance of glioblastoma (GBM) cells and NSCs. First, treatment of FGF1 increases AurA expression in human GBM cell lines. Second, using fluorescence-activated cell sorting, we observed that F1BGFP reporter facilitates the isolation of F1BGFP(+) GBM cells with higher expression levels of FGFR and AurA. Third, both FGFR inhibitor (SU5402) and AurA inhibitor (VX680) could down-regulate F1BGFP-dependent AurA activity. Fourth, inhibition of AurA activity by two different AurA inhibitors (VX680 and valproic acid) not only reduced neurosphere formation but also induced neuronal differentiation of F1BGFP(+) GBM cells. Fifth, flow cytometric analyses demonstrated that F1BGFP(+) GBM cells possessed different NSC cell surface markers. Finally, inhibition of AurA by VX680 reduced the neurosphere formation of different types of NSCs. Our results show that activation of AurA kinase through FGF1/FGFR signaling axis sustains the stem cell characteristics of GBM cells.

PP01.189

Characterization of the Toxic Compounds From the Androctonus Mauretanicus Scorpion Venom

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Abstract: Scorpion venoms are very complex mixtures of molecules, most of which are peptides displaying different kinds of biological activity. Indeed, these peptides specifically bind to a variety of pharmacological targets, in particular ionic channels located in prey tissues, resulting in neurotoxic effects. Toxins modulating Na+, K+, Ca2+ and Cl(-) currents have been described in scorpion venoms. In this work, we have used several specific antibodies raised against the most lethal scorpion toxins already described to screen the Moroccan scorpion Androctonus mauretanicus venom in order to characterize new compounds.

This immunological screening was also implemented by toxicity tests in mice and with mass spectrometry study, providing new informations on the molecular composition of this venom. In fine, we were able to determine the molecular masses of 70-80 different compounds. According to the immunological data obtained, many toxins cross-react with three sera raised against the most lethal alpha-toxins found in North African scorpion venoms, but not at all with those raised against the main beta-toxins from South and North American venoms. Some of the previously described toxins from Androctonus mauretanicus venom could thus be detected by combining immunological tests, toxicity in mice and molecular masses.

PP01.190

Mechanism-Based Inhibitor Design Towards the Catalytic Hydrogen Bond Network of Glutaminyl Cyclases

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Abstract: Glutaminyl cyclases (QCs) are zinc-containing enzymes that catalyze N-terminal pyroglutamate formation of numerous bioactive proteins and peptides. The enzymes are emerging drug targets for the treatment of Alzheimer's disease and some inflammatory disorders. In this poster, we describe that a catalytic H-bond network is highly conserved in the QC family. Disruption of the H-bond network by single amino acid mutations led to inactivation of enzymatic activity without significant change in the active-site conformation. Moreover, we found that several divalent metal ions were able to strongly inhibit the enzymatic activities of QCs, particularly Cd^{2+}, Zn^{2+}, Cu^{2+}, and Co^{2+} in the range of 5-500 ×M concentrations. Enzyme kinetic analysis indicated that the metal ions strongly reduced the turnover rate (k_{cat}) of QCs, suggesting that the bound metal ions disrupted the H-bond network of the enzymes. X-ray crystallographic studies revealed that one metal ion bound to the QC active site when QC crystals were soaked in 1 mM metal ions, while two or three metal ions bound to the active site when the crystals were soaked in 5 mM metal ions. Notably, the second and third bound metal ions disrupted or weakened the catalytic H-bond network of QCs by binding to the residues that make up the H-bond network, consistent with the finding from enzyme kinetic analysis. These results strongly suggest that the H-bond network is a good target of QC inhibitors, thus providing a new direction of drug discovery.

PP01.191

Evaluating Mutant Specific Potency of Histone Deacetylase Inhibitors on Niemann-Pick Type C1 Chimeras

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Abstract: Niemann Pick Type C1 (NPC1) is a fatal inherited neurodegenerative lysosomal storage disorder caused due to mutation in *npc1* gene that encodes for a late endosomal-lysosomal (LE/Ly) resident trans-membrane protein NPC1. It is characterized by abnormal accumulation of cholesterol and other glycosphingolipids such as GM2 and GM3 gangliosides in the LE/Ly compartments of the cells in multiple tissue types. It is manifested by cognitive impairment, ataxia and death most often in childhood. We have previously reported that histone decetylase inhibitor (HDACi) corrects the NPC1 phenotype and clears cholesterol accumulation in LE/Ly compartment of patient derived skin fibroblast. Our *in-vitro* data indicated that these inhibitors are ineffective on NPC1*null* cells, but increased and stabilized NPC1 protein expression in most prevalent I1061T mutant cells. However, the effects of HDACi treatment on other NPC1 mutations remained unclear. There are more than 300 different disease causing NPC1 mutations reported in clinical patients. Hence, with the aim to identify all NPC1 mutations treatable by HDACi a 384-well plate screen was designed. The NPC1*null* human osteosarcoma cell line (U2OS_shNPC1) was generated for the screen. NPC1*null* cells were transfected with 81 different lentivirus based mutant NPC1 and GFP coexpression system by reverse transfection in high throughput format to express the mutated NPC1 protein over $NPC1^{null}$ background. Using this system, we tested the effects of two HDACi's: Vorinostat and LBH589 on transiently transfected 81 NPC1 mutations simultaneously in clearing cholesterol accumulation. The results will help us in determining which NPC1 mutations in patient will benefit by these HDACi treatment.

PP01.192

Insulin Receptor Internalization Route and Dynamics in Pancreatic Beta-Cells and Muscle Cells

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Abstract: The insulin receptor (IR) is one of the most studied receptor tyrosine kinases, but fundamental aspects of its biology remain unclear. IR internalization upon insulin binding enables IR signaling from endosomal compartments and is a possible mechanism to selectively activate different downstream signaling pathways. It is suggested that IR endocytosis is mediated by clathrin in hepatocytes but by caveolin1 (Cav1) in adipocytes, in which membrane caveolae domains also play important roles in IR signaling. However, internalization mechanisms were

unclear in pancreatic beta cells and muscle. We developed novel inter-domain-tagged IR biosensors, wherein fluorescent proteins were placed at the extracellular region in between two functional domains, to illustrate above questions. Our fusion proteins retained their basic insulin-stimulated signaling and mimicked the localization of endogenous IRs. In beta cells, we mapped the trafficking of IRs to Cav1, flotillin1 and Lamp1 positive compartments, bypassing clathrin, Rab5a, Rab7, Rab11a, or Rab4a positive compartments. Multiple methods of inhibiting caveolin-1 significantly reduced Erk, but not Akt activation. Similarly, inter-domain tagged IR co-localize with caveolin, but not clathrin, in C2C12 myoblasts. Preliminary results also showed that the surface dynamics and lifetime of inter-domain tagged IR differs at different insulin treatment time points. We further examined the muscle specific and insulin dependent IR dynamics and internalization route, and how they potentially affect IR signaling. We expect that these novel approaches to live-cell imaging of IR dynamics will be important for the elucidation of insulin signaling biology.

PP01.193

Up-Regulation of EGFR Mediated by CUG2, a Novel Oncogene, Confers Anti-Cancer Drug in Human Lung Cancer Cells

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Abstract: Our previous study showed that cancer upregulated gene (CUG) 2, a novel oncogene induced anticancer drug resistance and other studies also reported that overexpression of EGFR was involved in the same pathophysiological phenomena. We thus explore whether CUG2 exerts anticancer drug resistance through enhancement of EGFR expression in this study. We found that CUG2 induced up-regulation of EGFR and doxorubicin resistance in human lung cancer A549 cells. A549 control cells produced a drastic amount of reactive oxygen species (ROS) upon treatment with doxorubicin while CUG2 expression reduced ROS by enhanced expression of anti-oxidant proteins such as Mn-SOD, FOXO1, and FOXO4. Furthermore, CUG2 expression increased NF-κB activity, which eventually turned on synthesis of multidrug resistance genes such as MRP2, and BCRP. However, suppression of EGFR expression dramatically enhanced ROS by the reduction of anti-oxidant proteins and NF-κB activity, resulting in sensitization to doxorubicin-induced apoptosis. Taken together, upregulation of EGFR mediated by CUG2 confers anti-cancer drug resistance through the increase of anti-oxidant protein levels and NF-κB activity.

PP01.194

SUMO Specific Protease 2 (SENP2) Mediates the Effect of Leptin on Lipid Metabolism in Myotubes

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Abstract: Leptin, a hormone secreted from adipocytes, plays a key role in the regulation of appetite, energy expenditure and adiposity. Leptin decreases appetite through its actions in the hypothalamus. Leptin, however, also increases energy expenditure via peripheral actions in skeletal muscle. Generally, leptin binds to the leptin receptor (Ob-R), and induces activation of janus kinase 2 (JAK2) and subsequent phosphorylation of signal transducer and activator of transcription 3 (STAT3). Chronic leptin treatment increases fatty acid oxidation (FAO) in muscle, which is related to increase in the mRNA levels of FAO-related genes such as *cpt1*, *mcad* and *ucp2*. The mechanism, by which expression of FAO-related genes is increased in response to leptin, has not been elucidated. We have reported that SUMO specific protease2 (SENP2) increases FAO-related gene expression through desumoylation and subsequent activation of PPARs in muscle. In this study, we examined the effects of leptin treatment on fatty acid metabolism in C2C12 myotubes. Interestingly, SENP2 mRNA levels were increased by leptin, which was dependent on leptin receptor and STAT3. Importantly, knock-down of SENP2 significantly suppressed leptin-induced increase of FAO and FAO-related gene expression. These results suggest that SENP2 plays an important role in the regulation of fatty acid metabolism by leptin in skeletal muscle.

PP01.195

Transcriptional Activity of Deltalactoferrin Controled by a Crosstalk Between Several Posttranslational Modifications

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Abstract: Deltalactoferrin (DLf) is a transcription factor with antitumor activities. Posttranslational modifications such as *O*-GlcNAcylation and phosphorylation, efficiently modulate its transcription factor activity and stability. Recently we first showed that DLf is modified by SUMO-1 and mapped the five SUMO sites. In a second time we produced a series of mutants for which only one site was preserved and a null-mutant in which all five SUMO sites were invalidated. We showed that all lysine residues were SUMO acceptors and that K13, K308 and K379 were the main SUMO sites. We studied the impact of SUMOylation on DLf activity and showed that SUMOylation negatively regulated the transactivation function of DLf. In a next time we investigated the crosstalk between different posttranslational modifications. We showed that K379 which is either ubiquitinated or SUMOylated,

is a pivotal site for the control of DLf stability. We also showed that SUMOylation competes with ubiquitination and protects DLf from proteosomal degradation by positively regulating its stability. We demonstrated that K13 is the main acetylation site and that favoring acetylation at K13 reduced SUMOylation and increased DLf transcriptional activity. Collectively, our results indicate that multi-SUMOylation occurs on DLf to repress its transcriptional activity. Reciprocal occupancy of K13 by either SUMO-1 or an acetyl group may contribute to the establishment of finely regulated mechanisms to control DLf transcriptional activity. Moreover, competition between SUMOylation and ubiquitination at K379 coordinately regulates the stability of DLf toward proteolysis. Therefore SUMOylation of DLf is a novel mechanism controlling both its activity and stability.

PP01.196

Age-Related Metabolic Alterations in Accelerated Aging Klotho (-/-) Mice

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Abstract: Klotho is a transmembrane protein implicated in a variety of premature aging phenotype processes including phosphate (Pi) and vitamin D metabolism. We observed that Klotho (-/-) importantly displayed lower lifespan and motor coordination than control mice. Age-related metabolic changes were determined in the kidney of aged (8-12 week) Klotho (-/-) mice. Klotho (-/-) mice were showed decreased levels of anti-oxidants and purine base and altered kidney function that contribute to premature CKD in the Klotho (-/-) mice. Intriguingly, we identified the metabolic pathways, reduced glutathione (GSH) metabolism and pentose and glucoronate interconversions. These new metabolic pathways may provide new understanding for the CDK-associated mechanisms under aging klotho (-/-) mice. (This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government MSIP (No.2008-0062283)).

PP01.197

2-Methoxycinnamaldehyde Inhibits the TNF-α-Induced Proliferation and Migration of Human Aortic Smooth Muscle Cells

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Abstract: The abnormal proliferation and migration of vascular smooth muscle cells (VSMCs) are crucial event for the development of atherosclerosis, and TNF-α is actively involved in this process by enhancing the proliferation and migration of VSMCs. 2-methoxycinnamaldehyde (MCA) is a natural compound of *Cinnamomum cassia*. While 2-hydroxycinnamaldehyde (HCA), another compound from *Cinnamomum cassia*, is widely studied regard to its antitumor activity, MCA has not attracted researchers' interest because of its mild toxicity on cancer cells and its still-unknown mechanism of action. In this study, we examined the effects of MCA on TNF-α-induced HASMC (human aortic smooth muscle cell) proliferation and migration. We showed that MCA inhibited TNF-α induced cell proliferation by reducing the levels of cyclin D1, cyclin D3, CDK4 and CDK6 and increasing the levels of cyclin-dependent kinases p21 and p27 without resulting in cell cytotoxicity. Furthermore, MCA decreased the level of secreted MMP-9 by inhibiting *MMP-9* transcription. Unexpectedly, MCA did not affect the TNF-α-induced levels of MAPKs. However, by showing that MCA potently inhibited the degradation of IκBα and the subsequent nuclear translocation of NF-κB, we demonstrated that MCA acts through the NF-κB signaling pathway. MCA also effectively inhibited the PDGF-induced HASMC migration. Taken together, these observations suggest that MCA has the potential for use as an anti-atherosclerotic agent.

PP01.198

Retinoic Acid Signaling Plays Critical Roles in Epicardial-Regulated Programs During Late Heart Development

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Abstract: The epicardium, a layer of cells covering the surface of the heart, is essential for the development of the coronary vasculature and the myocardium. In the developing heart, epicardial cells become motile through epithelial-to-mesenchymal transition (EMT) and migrate into the myocardium where they differentiate into vascular smooth muscle cells or fibroblasts to stabilize immature endothelial tubes. Besides its role as a source of progenitors for vascular cells, the epicardium also secretes several paracrine factors, including retinoic acid (RA). RA signaling has been proposed to promote myocardial growth and regulate the formation of the coronary vessels, though very few genetic models support this paradigm and the mechanisms involved remain poorly understood. Here we investigate the role of RA in the development of coronary vessels by using several genetic and pharmacologic models of dysregulated RA formation in the developing mouse. Our results provide evidence that appropriate RA signaling is essential for the formation of the coronary vasculature. RA-excess results in a thin myocardium and impaired remodeling of the coronary vasculature; meanwhile, RA-deficiency causes a loss the coronary vascular hierarchy. To further understand the mechanisms responsible for the regulation of coronary development by RA, we studied the effect of RA *in vitro* by examining the differentiation of primary fetal epicardial cells. These studies suggest that RA controls the EMT and migration of the epicardial-derived mural cell precursors. Based on these findings, we propose that the angiogenic remodeling of the coronary vasculature could be controlled through the manipulation of RA-signaling pathways.

PP01.199

Pathogenic Role of CXCL9 E CXCL10 Expressing Monocytes Derived-Dendritic Cells in Experimental Cerebral Malaria

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Abstract: Monocytes (MOs), macrophages and dendritic cells (DCs) are heterogeneous cell populations that have critical role in tissue repair, sensing presence of invasive microorganisms and initiating protective immune responses. Recent studies have defined new markers that allows the distinction of inflammatory monocytes from monocyte derived dendritic cells (MO-DCs), however the contribution of these cells to neuroinflammation during ECM have not been explored. Here we show that infection with *Plasmodium berghei* ANKA (*Pb*A) promotes replacement of splenic inflammatory monocytes and conventional DCs by monocyte-derived dendritic cells (MO-DCs), which are $CD11c^+CD11b^+F4/80^+DC\text{-}SIGN^+Ly6c^+$. These cells are highly responsive to IFNg being a main source of CXCL9 as well as CXCL10. Importantly, the CXCL9/10 MO-DCs emerge in the brain in a CCR5-dependent manner, coinciding with $CD8^+$ T cell influx and development of the lethal neuropathological syndrome. Thus, we provide evidences that the MO-DCs induced by *Pb*A infection play a central role in initiating immune responses and mediating the development of experimental cerebral malaria (ECM). Taken together, we would like to demonstrate that after differentiation, the CXCL9/10 MO-DCs not only have an important role in activating both $CD4^+$ T and $CD8^+$ T cells in the spleen, but also migrate to the CNS in a CCR5-dependent manner, where they attract/activate $CD8^+$T cell leading to the development of ECM.

PP01.200

PCTK3/CDK18 Regulates Cell Migration by Negatively Modulating the FAK1 Activity

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Abstract: PCTAIRE kinase 3 (PCTK3) is a member of the cyclin dependent kinase (CDK) family, but its physiological function remains unknown. We previously revealed that PCTK3 is activated through interaction with cyclin A and phosphorylation by PKA. Furthermore, we also found that PCTK3-knockdown HEK293T cells showed morphological changes involving actin accumulation at lamellipodia, suggesting that PCTK3 is involved in the regulation of actin reorganization. However, the downstream pathways of PCTK3 have been less understood. In this study, we investigated the physiological function and the downstream signal transduction molecules of PCTK3. First, to assess whether PCTK3 regulates cell migration, the *in vitro* scratch wound healing assay was performed. PCTK3-knockdown HEK293T cells exhibited an increase in cell motility as compared with control cells. Additionally, we demonstrated that phosphorylation of cofilin, an actin depolymerizing factor, is increased in PCTK3-knockdown cells. Because cofilin depolymerizing activity is regulated by Rho GTPases such as RhoA and Rac1, we next investigated whether PCTK3 affects the activities of RhoA and Rac1. PCTK3 knockdown led to RhoA activation and Rac1 inactivation in HEK293T cells, indicating that PCTK3 modulates cell migration via the regulation of RhoGTPase activity. Finally, we found that the activity of focal adhesion kinase 1 (FAK1), which is activated during early cell adhesion, was suppressed by PCTK3, suggesting that PCTK3 might act as a negative regulator of FAK1.

PP01.201

eIF4E-Dependent Regulation of MRNA Translation Controls Mouse Embryonic Stem Cell Self-Renewal

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Abstract: Translational control has been documented primarily at the initiation step. The eukaryotic translation initiation factor 4E (eIF4E) is the mRNA 5′ cap-binding protein, which together with the scaffolding protein eIF4G and the RNA helicase eIF4A form the eIF4F complex that recruits the mRNA to the ribosome. 4E-BPs are a family of small translational inhibitors (4E-BP1, 2 and 3 in mammals), which bind to and suppress the activity of eIF4E by blocking its association with eIF4G, and consequently the assembly of the eIF4F complex. We recently reported that 4E-BP-dependent translational control plays an important role in the regulation of transcription factors-induced reprogramming (*Tahmasebi, 2014; Cell Stem Cell 1;14(5):606-16).* Our new findings indicate that 4E-BP1/2 DKO ES cells are defective in maintaining ES cell self-renewal. Using ribosome profiling we identified transcripts, which show enhanced translation in 4E-BP-DKO ES cells. Using western blotting, we validated the expression of proteins encoded by mRNAs translationally upregulated in 4E-BP1/2 DKO ES cells. The function of these proteins in ES cells self-renewal is under investigation.

PP01.202

Role of the Metabolic Haloacid Dehalogenase-Type Phosphatase AUM for Cell Migration and Cell Spreading

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Abstract: We have identified AUM (gene annotation, phosphoglycolate phosphatase), a previously unexplored

phosphatase of the haloacid dehalogenase (HAD) superfamily. RNA interference-mediated AUM depletion accelerates and enhances cell adhesion on different integrin ligands but also increases cell migration after treatment with the RTK ligand EGF. To investigate a potential role of AUM for integrin- and RTK-dependent actin cytoskeletal reorganization, AUM-depleted and control shRNA cells were seeded on fibronectin and stimulated with EGF. Interestingly, EGF treatment led to an enhanced formation of circular dorsal ruffles (CDR) in AUM-depleted cells. CDR are Src-dependent, actin-based structures that promote integrin internalization and recycling, and are thus involved in the regulation of cell adhesion and cell migration on extracellular matrix molecules. By using pharmacological inhibitors we analyzed molecular signals that control AUM-dependent CDR-formation. We could show that phospholipase C (PLC) mediated activation of proteinkinase C (PKC) leads downstream of Src signaling to effects on CDR-formation in an AUM-dependent manner. Furthermore we could demonstrate that AUM acts as a metabolic phosphatase by interfering with the glycerolipid/free fatty acid cycle (Kennedy Pathway). Mass spectrometric based lipodomics analysis (LC-MS/MS) elucidates the accumulation of the membrane lipid phosphatidylserine (PS) in AUM-depleted cells. PS is a negatively charged phospholipid and is implicated in signaling pathways through the recruitment and binding of signal molecules like PLC and PKCs. The accumulation of PS in the plasma membranes of AUM-depleted cells leads to a pre-assembly and activation of signaling molecules and may explain the acceleration and increase in cell adhesion and migration.

PP01.203

Defective Receptor Protein Tyrosine Kinase PTK7 Enhances Oncogenic and Metastatic Abilities of ESCC Cells

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Abstract: A defective receptor protein tyrosine kinase, protein tyrosine kinase 7 (PTK7), is upregulated in various cancers including esophageal squamous cell carcinoma (ESCC). Here, we have explored the relationship between PTK7 and ESCC and effect of PTK7 expression on oncogenic and metastatic potentials of ESCC cells. Increased PTK7 expression correlates with poor prognosis of ESCC patients and enhances proliferation, survival, migration, and invasion of ESCC cells. PTK7 knockdown reduces gelatin degradation and MMP-9 secretion in cultures of ESCC TE-10 cells and shows reduced levels of *MMP9* mRNA using real-time RT-PCR and luciferase reporter assays. PTK7 knockdown decreases not only phosphorylation of NF-κB, IκB, ERK, and JNK, but also nuclear localization of NF-κB and AP-1 consisting of c-Fos and c-Jun. Activation of AP-1 and NF-κB requires PTK7-mediated activation of tyrosine kinases, including at least Src family kinases. In addition, NF-κB activation by PTK7 involves the PI3K/Akt signaling pathway. PTK7-mediated upregulation of *MMP9* was also observed in other ESCC cell lines and in three-dimensional cultures of TE-10 cells, and MMP-9 expression positively correlated with PTK7 expression in ESCC tumor tissue. These findings demonstrate that PTK7 upregulates *MMP9* through activation of AP-1 and NF-κB and, thus increases invasive properties of ESCC cells.

PP01.204

Selection of DNA Aptamers Against H5N1 and H5N2 Subtypes of Influenza Virus A

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Abstract: Highly pathogenic avian influenza A virus of the H5N1 subtype (HPAI H5N1) is one of the most virulent influenza viruses in human and chicken and cause a mortality rate of about 60% for human and 100% for chicken. Since the late 1990s, HPAI H5N1 viruses have devastated the poultry industry of numerous countries in the Eastern Asia. To date, HPAI H5N1 has spread from Asia to Europe, Africa, and the Middle East, resulting in the death of hundreds of millions of poultry. Recently, HPAI H5N2 also raised concern in China and Taiwan. A cheaper and easy instant confirmation tool is helpful for the infield detection and differentiation of HPAI subtype H5 from the other major avian infectious diseases. We applied recently developed technique systematic evolution of ligands by exponential enrichment (SELEX) to select DNA aptamers against the recombinant H5N1 hemagglutinin globular domain. The selected candidate aptamers were competed with α-2, 3-linked sialic acid to get the sialic acid-specific aptamers. The selected aptamer, SAC-11, bound specifically to the influenza A/Vietnam/1204/2004 (H5N1) and A/duck/Yunlin/2004 (H5N2) viruses but not recognized the other two major avian viruses infectious bronchitis virus and newcastle disease virus. In this study, we discovered an HPAI subtype H5 specific aptamer which has potential for use in the rapid detection of HPAI virus.

PP01.205

TMEM16A and Myocardin Form a Positive Feedback Loop That Is Disrupted by KLF5 During Ang II-Induced Vascular Remodeling

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Abstract: The TMEM16A protein is an important component of Ca^{2+}-dependent Cl^- channels (CaCCs) in vascular smooth muscle cells (VSMCs). A recent study showed that TMEM16A inhibits angiotensin II-induced proliferation in rat basilar smooth muscle cells. However, whether and how TMEM16A is involved in vascular remodeling characterized by VSMC proliferation remains largely unclear. In this study, luciferase reporter, Western blotting, and qRT-PCR assays were performed. The results suggested

that myocardin promotes TMEM16A expression by forming a complex with serum response factor (SRF) on the TMEM16A promoter in human aortic smooth muscle cells (HASMCs). In turn, upregulated TMEM16A promotes expression of myocardin and VSMC marker genes, thus forming a positive feedback loop that induces cell differentiation and inhibits cell proliferation. Angiotensin II inhibits TMEM16A expression via Krüppel-like factor 5 (KLF5) in cultured HASMCs. Moreover, in vivo experiments show that infusion of angiotensin II into mice causes a marked reduction in TMEM16A expression and vascular remodeling, and angiotensin II-induced effects are largely reversed in KLF5 null ($KLF5^{-/-}$) mice. KLF5 competes with SRF to interact with myocardin, thereby limiting myocardin binding to SRF and the synergistic activation of the TMEM16A promoter by myocardin and SRF. Our studies demonstrated that angiotensin II induces KLF5 expression and facilitates KLF5 association with myocardin to disrupt the myocardin-SRF complex, subsequently leading to inhibition of TMEM16A transcription. Blocking the positive feedback loop between myocardin and TMEM16A may be a novel therapeutic approach for vascular remodeling.

PP01.206

Involvement of miR-29a in Arteriosclerosis Acceleration Mediated by KLF5

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Abstract: Rationale—Coronary artery disease is the leading cause of death in the world. The regulation of vascular smooth muscle cell (VSMC) proliferation is an important issue due to its major implications for the prevention of pathological vascular conditions. Objective—We examined the role of miR-29a and its mechanism of action in atherogenesis in transgenic mice. Methods and Results— We found that circulating levels of miR-29a are significantly downregulated in patients with coronary artery disease. To further detect the miR-29a mechanism of action in atherogenesis, $ApoE^{-/-}$, $ApoE^{-/-}$miR-29a$^{+/+}$ and $ApoE^{-/-}$KLF5 $^{/}$ mice were fed standard chow or a Western diet. After 12 weeks, serial echocardiography was performed to measure aortic plaque formation. Feeding mice a Western diet markedly increased the cholesterol and triglyceride levels, the aortic plaque area, intima-media thickening, and the VSMC/Mϕratio. These characteristics were increased in the VSMC-specific miR-29a-transgenic mice and decreased in the VSMC-specific KLF5-knockout mice. In the aorta, miR-29a overexpression increased VSMC proliferation and the cholesterol and triglyceride levels by increasing the expression of KLF5. VSMC-specific KLF5 knockout significantly ameliorated atherosclerosis and VSMC proliferation. Further mechanistic studies showed that miR-29a reduced the expression of Fbw7/CDC4 protein by targeting its 3'-UTR, subsequently leading to increased KLF5 stability through the decreased ubiquitination of KLF5 by Fbw7/CDC4. Conclusions—miR-29a targets Fbw7/CDC4 and increases KLF5 stability by reducing KLF5 ubiquitination by Fbw7/CDC4.

PP01.207

Identification and Characterization of Soybean ER Membrane-Bound Protein Disulfide Isomerase Family Protein, GmPDIL7

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Abstract: Introduction: Most proteins synthesized in the endoplasmic reticulum (ER) have disulfide bonds, which play an important role for conformation stability and function of proteins. In eukaryotic cell, there are protein disulfide formation pathways such as Protein Disulfide Isomerase (PDI)-ER oxidoreductin1 (Ero1) system in the ER lumen. **Objectives:** The objectives are identification of a novel soybean ER membrane-bound PDI family protein, GmPDIL7 and determination of its enzymatic properties. **Materials and Methods:** The *GmPDIL7* cDNA was cloned by RT-PCR. Recombinant GmPDIL7 was expressed in *E. coli* and purified. Thiol oxidation activity was measured using decapeptide containing two cysteine residue. Oxidation of GmPDIL7 by GmERO1 was measured by assay coupled with GSH and NADPH. Oxidative refolding activity was measured by refolding of reduced and denatured RNaseA. **Results:** GmPDIL7 possesses a putative N-terminal signal sequence, a thioredoxin domain with an active-center motif (CGHC), and a putative C-terminal transmembrane region. GmPDIL7 ubiquitously expressed in soybean tissues. GmPDIL7 was revealed to localize in the ER membrane by cell fractionation and confocal microscopy observation of a specimen immunostained with the anti-GmPDIL7 serum. The redox potential of recombinant GmPDIL7 was -187.4mV, showing that GmPDIL7 could oxidize unfolded proteins. Thiol oxidation activity of GmPDIL7 was similar to those of other soybean PDI family. GmPDIL7 was well oxidized by GmERO1. However, oxidative refolding activity of GmPDIL7 was lower than other soybean PDI family. **Conclusion:** It is predicted that GmPDIL7, which is the ER membrane protein, mainly plays a role as an oxidase in oxidative protein folding.

PP01.208

Molecular Mechanism of Cooperative Oxidative Folding by Plant Ero1 and Multiple ER Protein Thiol Disulfide Oxidoreductases

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Abstract: Introduction: Most proteins produced in the endoplasmic reticulum (ER) of eukaryotic cells are folded via disulfide formation (oxidative folding). Oxidative folding is catalyzed by ER protein thiol disulfide oxidoreductases (ER oxidoreductases) such as protein disulfide isomerase (PDI). In yeast and mammals, ER oxidoreductin (Ero1) supplys oxidizing equivalents to the active centers of PDI. **Objectives:** In this study, we tried to clarify the molecular mechanism of oxidative folding in plant with an *in vitro* reconstituted system with soybean Ero1 (GmERO1a) and soybean ER oxidoreductases. **Materials and Methods:** Recombinant GmERO1a, five soybean ER oxidoreductases and their active-center mutants were prepared with *Escherichia*

*coli*expression system. Oxidation of ER oxidoreductases by GmERO1a was measured using a coupled assay following the consumption of NADPH by glutathione reductase. Oxidative refolding activity was assayed with reduced RNase A as a substrate. **Results:** GmERO1a oxidized multiple soybean ER oxidoreductases, in contrast to mammalian Ero1 having a high specificity for PDI. GmPDIM associated *in vivo* and *in vitro* with GmPDIL-2. GmPDIL-2 synergistically accelerated oxidative refolding by GmPDIM and GmERO1a. In this process, GmERO1a preferentially oxidized the active center in the **a'** domain of GmPDIM. The disulfide bond was shown to be transferred to the active center of the **a** domain of GmPDIM and then to the active centers of the **a** or **a'** domain of GmPDIL-2. **Conclusion:** The relay of an oxidizing equivalent produced by Ero1 from one ER oxidoreductase to another may play an essential role in cooperative oxidative folding by multiple ER oxidoreductases in plants.

PP01.209

O-GlcNAcase Is Necessary for Learning and Memory and Synaptic Plasticity

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Abstract: O-GlcNAcylated proteins are abundant in brain and associated with neuronal functions and neurodegenerative diseases. Although several studies have reported the effect of aberrant regulation of O-GlcNAcylation on brain function, the roles of O-GlcNAcylation in synaptic function is not clear. To understand the effect of aberrant O-GlcNAcylation on brain, we used *Oga+/-* mice, which have an increased level of O-GlcNAcylation. We found that *Oga+/-* mice exhibited impaired spatial learning and memory. Consistent with this results, *Oga+/-* mice have defect in hippocampal synaptic plasticity. *Oga* heterozygosity causes impairment of both long-term potentiation (LTP) and long-term depression (LTD). These results demonstrate a role for hyper-O-GlcNAcylation in learning and memory

PP01.210

Proteomic Mapping of the Interacting Proteins With STEAP1 May Determine the Mechanisms That Regulate Oxidative Stress by STEAP1

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Abstract: Six transmembrane epithelial antigen of the prostate 1 (STEAP1) is a cell surface antigen with metalloreductase activity that is highly expressed in numerous carcinomas of the prostate and bladder, and also Ewing sarcoma (EWS). STEAP1 induces reactive oxygen species (ROS) in EWS and associated with tumour proliferation and invasion. However, the mechanism that links STEAP1 to regulation of ROS is unknown. In this study we used several mass spectrometry (MS) based approaches to identify potential interacting proteins with STEAP1 that may mediate increased ROS generation in sarcoma cell lines. Anti-STEAP1 antibody was conjugated with horseradish peroxidase (HRP) to label surface molecules in proximity of STEPA1 protein (200–300 nm) with biotin-phenol. Then the purified biotinylated proteins were identified and quantified by MS analysis. We also used cross-linking/immunoprecipitation method to establish covalent linkage between STEAP1 and interacting proteins which subsequently purified by immunoprecipitation of the STEPA1 protein complex and analysed by MS. These approaches were combined with MS quantification methods such as tandem mass tag (TMT) labelling and stable isotope labeling by amino acids in cell culture (SILAC) to improve the specificity of proteomic mapping of the interacting proteins with STEAP1. The identified proteins in these approaches may mediate STEAP1 functions. Although, further mechanistic analysis is required to determine the mechanism.

PP01.211

Controllable Protein Trans-Splicing by Two-Intein Hybrids

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Abstract: Protein splicing is a spontaneous post-translational process, by which an intervening polypeptide (called intein), catalyzes its own excision from the flanking polypeptides (called exteins), as well as ligation of the exteins. Intein mediated protein splicing is recognized as a potentially general tool to control the activity of proteins in living cells. In this study, we constructed hybrid-two-intein system to control the ligation of two model proteins by an artificial switch (large fragment of two split inteins). First by using our previous split inteins pairs without cross reaction, we have got 4 out of 9 combinations with relatively high trans-splicing activities. We will further test their splicing activities in vitro. These hybrid-two-inteins design could precisely control the ligation of two proteins or the activity of one intein by the artificial switch with the minimum exogenous amino acids. If the activity of the intein can be switched at will, then this regulatory element could be used to control the function of any other protein.

PP01.212

Post-Translational Modifications of p62 Modulate Ub-Dependent Selective Autophagy via Biochemically Distinct Mechanisms

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Abstract: Autophagy is an evolutionarily conserved cellular metabolism process that traffics cytosolic proteins and organelles to lysosomes for degradation and recycling. This process plays a critical role in maintaining cellular homeostasis and its malfunction has been implicated in the pathogenesis of multiple human diseases including cancer, diabetes and neurodegeneration. p62/SQSTM1 is an essential receptor protein in the selective autophagy process. p62 binds to ubiquitinated substrates through its C-terminal ubiquitin-associated (UBA) domain and assmble them into aggregates via self-oligomerization. The aggragated material is then packed into autophagosomes and trafficked to lysosomes for degradation. Recent studies from our own lab and others have found that proteotoxic stress, but not nutrient starvation, induces phosphorylation at two specific sites S405 and S409 within the UBA domain. Phosphorylation at either site leads to enhanced binding of UBA to ubiquitin and promotes the clearance of ubiquitinated protein aggregates. We conducted biochemical and structural studies to delineate the molecular mechanism of how phosphorylation at S405 and S409 can lead to enhanced interction between UBA domain and ubiquitin. Our data reveal that phosphorylation at S405 enhances the UBA-ubiquitin interaction by likely forming direct electrostatic interactions with ubiquitin. However, S409 phosphorylation employs a different mechanism to enhance Ub binding. Phosphorylation mimicking mutation S409E destabilizes the UBA dimer interface and promotes its transition to the monomeric form suitable for Ub binding. Furthermore we have identified post-translational modification on lysine residues within the UBA domain that affect Ub binding as well. The mechanism of such modifications are yet to be studied.

PP01.213

Carbamazepine Confers Protective Effects on Pancreatic β Cells to Reduce Type 1 Diabetes in Non-Obese Diabetic Mice

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Abstract: Pancreatic β cells are selectively destroyed by the host immune system in type 1 diabetes. Previous work from our laboratory found that the use-dependent sodium channel blocker, carbamazepine, protects β cells from inflammatory cytokines in vitro. To validate these results, female non-obese diabetic (NOD) mice were fed LabDiet 5053 alone (n = 20) or supplemented with 0.5% w/w carbamazepine (n = 22). Development of diabetes as determined by fasting blood glucose showed a reduction in incidence from 80% to 40% at 25 weeks of age between control and drug treated animals. Serum carbamazepine levels measured at experimental endpoint (14.98 4 3.19 ×M) were consistent with the concentrations used to identify the compound in vitro (~8.5 ×M). No significant differences in insulitis (infiltration of immune cells into the pancreatic islets) were observed between control and drug treated animals. Carbamazepine treated mice were found to have increased insulin-positive β cell area as compared to controls. A glucose tolerance test of a pre-diabetic cohort of mice revealed a statistically significant difference in AUC of carbamazepine treated (474.6 4 21.8) versus control mice (676.2 4 40.7; p < 0.01). Interestingly, changes in T cell composition and activity were also not observed. Taken together, carbamazepine reduces the development of type 1 diabetes in NOD mice. Evidence suggests that this observation may be due to direct protective effects on β cells and not on immune inhibition.

PP01.214

Regulation of Ubiquitination and Degradation of CRTC1 Transcriptional Coactivator by Salt-Inducible Kinase SIK1

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Abstract: CRTC1 is an essential transcriptional coactivator of CREB transcription factor, a master regulator of cell metabolism and physiology. Both CREB and CRTC1 are activated in response to extracellular and intracellular stimuli. Their activities are also subjected to stringent regulation by protein kinases and phosphatases. Salt-inducible kinase 1 (SIK1) activated by LKB1 kinase is one of the kinases that phosphorylate CRTC1 at S167 resulting in its cytoplasmic sequestration and inactivation. In this work we reported on a novel regulatory mechanism through which SIK1 phosphorylates CRTC1 at multiple sites to trigger ubiquitination and subsequent proteasomal degradation. The steady-state level of CRTC1 were dampened when SIK1 was overexpressed. Administration of proteasome inhibitor MG132 restored the level of CRTC1 protein, suggesting the involvement of proteasome in this process. Moreover, SIK1 induced CRTC1 ubiquitination, indicating that SIK1 destabilized CRTC1 through ubiquitin-proteasome pathway. Site-directed mutagenesis and mass spectrometry were used to define the multiple SIK1 phosphorylation sites in CRTC1. The impact of SIK1 regulation of CRTC1 activity on gluconeogenesis was also explored. Overexpression of CRTC1 significantly stimulated the transcription of gluconeogenic genes such as PEPCK-C and G6Pase, while this effect was effectively suppressed by SIK1. Besides, knockdown of endogenous CRTC1 protein completely blocked transcriptional activation of forskolin (FSK)-induced gluconeogenic genes, demonstrating the critical role of CRTC1 in this process. In summary, our findings reveal a novel cell signalling pathway for SIK1-dependent negative regulation of CRTC1 activity in which SIK1 phosphorylates and destabilizes CRTC1 leading to attenuation of transcriptional activation.

PP01.215

β-TrCP Targets Liver-Enriched and Membrane-Bound Transcription Factor CREB-H for Ubiquitination and Degradation

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Abstract: CREB-H is an endoplasmic reticulum-tethered bZIP transcription factor, which critically regulates lipid homeostasis and gluconeogenesis in the liver. CREB-H can be cleaved by site-1 and site-2 proteases to liberate an N-terminal activated form known as CREB-HΔTC, which translocates to the nucleus to activate target gene expression. In this study we identified and characterized β-TrCP to be an E3 ubiquitin ligase mediating polyubiquitination and proteasomal degradation of CREB-HΔTC, the physiologically active form of CREB-H. The degradation of CREB-HΔTC was mediated by lysine 48-linked polyubiquitination. Proteasome inhibition led to the accumulation of CREB-HΔTC. A DSGXS destruction box was identified in CREB-HΔTC and was also found to be conserved among orthologous proteins from different species. Disruption of this DSGXS destruction box resulted in stabilization of CREB-HΔTC. E3 ubiquitin ligase β-TrCP was found to be required for CREB-HΔTC polyubiquitination and degradation. Overexpression of wild-type but not dominant-inactive β-TrCP led to decreased expression and increased ubiquitination of CREB-HΔTC, whereas knockdown of β-TrCP had the opposite effect. Deletion of the DSGXS destruction box abolished the interaction of CREB-HΔTC and β-TrCP. Finally, CREB-H-mediated activation of the expression of key CREB-H target genes in liver-derived cell lines was found to be regulated by β-TrCP. Taken together, our work revealed a new signaling pathway that controls polyubiquitination and degradation of CREB-HΔTC. The rapid ubiquitination and degradation of CREB-HΔTC ensures transient and tightly regulated induction of its target genes in the liver. Supported by RGC (C7011-15R), NSFC (31500634) and SKYMRF (2011).

PP01.216

Potential Role of the Tripeptide, PGP, in Contributing to Inflammation Leading to Idiopathic Pulmonary Fibrosis

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Abstract: Fibrosis is a pathological condition resulting from chronic inflammation, which leads to abnormal deposition of collagen in many organs. Although collagen deposition is reversible and vital for wound healing, dysregulation and excess accumulation of connective tissue can lead to permanent damage and organ malfunction in kidney, liver and lung. Idiopathic Pulmunary Fibrosis (IPF) is a progressive and lethal disease characterized by accumulation of fibroblasts, connective tissue and severe architectural distortion of lung parenchyma. Several pathogenic mechanisms have been implicated in pathogenesis of IPF including: aberrant repair of injured epithelium, lack of apoptotic response of fibroblasts, fibroblast activation, epithelial-to-mesenchymal transition, and abnormal immune cell dysfunction. The focus of this research is on the regulation of a bifunctional enzyme called Leukotrience A4 Hydrolase, LTA4H, with pro and anti-inflammatory functions. While LTA4H catalyzes the conversion of leukotriene A4 to the proinflammatory mediator leukotriene B4, it has an aminopeptidase activity that has shown to degrade PGP, which has neutrophil chemotactic activity. Based on preliminary findings, we have been able to show that fibrotic lung tissue contains higher PGP content and reduced extracellular LTA4H, consistent with a possible role of PGP in development of IPF. In addition, we have demonstrated that LTA4H expression was much lower in fibroblasts from fibrotic lung. Future research will aim to test drugs that can block LTA4H hydrolase but not its peptidase activity, which may more effectively dampen the inflammatory response.

PP01.217

Ubiquitin Proteasome System-Mediated Degradation of Endothelin-Converting Enzyme-1c and Its Role in Colon Cancer Progression

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Abstract: Background. CK2 phosphorylates over 300 proteins regulating many cellular processes, most of them with relevance in cancer. ECE-1c is a metalloprotease involved in endothelin-1 synthesis which may act as a mitogen to promote cancer progression. We have previously demonstrated that the N-terminal end of ECE-1c is phosphorylated by CK2, which enhances its protein stability. In this work, we evaluated whether the CK2-dependent phosphorylation of ECE-1c prevents its ubiquitination and subsequent UPS-mediated degradation, as well as the role of protein interactors and its role in migration and invasion of colon cancer cells. Methodology. An ECE-1c-K6R mutant was designed and cloned in lentiviral and GST plasmids. Full-length ECE-1c-K6R protein stability was evaluated by cycloheximide treatment in CHO-K1 cells. Also, same mutant and cells were used for determining His-tagged ubiquitination by pull-down assay. Recombinant GST-tagged N-terminal of ECE-1c-K6R mutant bound to GSH-Agarose was incubated with cytosolic extracts of DLD-1 colon cancer cells and specifically interacting proteins were identified by MS. Migration and invasion were evaluated in full-length ECE-1c-K6R expressing DLD-1 cells by both transwell and matrigel assays, respectively. Results. (1) CK2 inhibition promoted ECE-1c UPS-dependent degradation, (2) specific proteins interacted with the N-terminal end of ECE-1c following CK2-phosphorylation, and (3) ECE-1c-K6R mutant improved migration and invasion of colon cancer cells. Conclusions. CK2-dependent phosphorylation at the N-terminal end of ECE-1c promotes its stability by blocking the UPS-mediated degradation, which improves migration and invasion of colon cancer cells. Acknowledgements. CONICYT Ph.D. Fellowships #21120181 (IN) and #21130753 (HH); FONDECYT grant #1120132 (JCT).

PP01.218

In Vitro Anticancer Activity of the Ethanolic Extracts of 53 Medicinal Plants Found in the Philippines

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Abstract: Plants have a long history in the treatment of various diseases including cancer. Current treatment cancer regimens include radiotherapy, surgery, and chemotherapy. Like most chemotherapeutic anticancer drugs, plant extracts work by targeting rapidly dividing cells by either impairing mitosis or causing cells to undergo apoptosis. In this study, crude ethanolic extracts of fifty-three Philippine medicinal plants were screened for *in vitro* anticancer activity against human colorectal cancer HCT116 using an MTT assay following a preliminary phytochemical screening and brine shrimp lethality assay. Highest cytotoxicity of HCT116 cell proliferation was observed using the extract of *Annona reticula.* The extracts of *Coleus blumei* and *Ficus septica* showed toxicity in a dose dependent manner. The extract of *Alium cepa* showed moderate cytotoxicity as well. Similarly, well-known edible plants, such as *Momordica charantia* and *Abelmoschus esculentus*, showed some inhibition of proliferation. Other plants that showed some proliferation inhibition properties include *Bauhinia integrifolia, Cananga odorata, Persea americana, Typhonium trilobatum,* and *Piper betle.* Based on our data, four of the 53 selected plant extracts showed cytotoxic activity against colon cancer cell line HCT116. It appears that traditional use of medicinal plants may serve as an initial guide for selection of plants for anticancer screening. In turn, the results of cytotoxicity screening may be used as *the starting point for succeeding tests to determine the component(s) responsible for cytotoxicity, as well us to unravel the likely pathways that are targeted by these components.*

PP01.219

Substantia Nigra Dysfunction in Schizophrenia; Evidence for Compromised Energy Metabolism

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Abstract: We have used Nano LC-MS/MS to establish the brain-site specific changes in schizophrenia related to normal controls, correlating with pathway involved and cross talk of regulated proteins in the region of substantia nigra. Present work focused not only on the conventional studies on cortex and hippocampus but also on the least explored aberrant expression of autopsied brain SN proteome, neglected despite its unique behavior in dopamine metabolism in schizophrenia . Using two dimensional electrophoresis (2DE) , Orbitrap mass spectrometry identification and validation of differential protein expression by western blot, the functional significance of the differentially expressed proteins has been interpreted utilizing improved enrichment statistical methods and an extensive collection of functional annotation pathways (IPA, PANTHER and STRING). Substantia nigra exhibits differential expression of twenty one proteins, enriched in several metabolic processes particularly protein such as ATP5H, GAPDH, MDHC, and PGAM1 of energy metabolism. Evidence of interplay among these proteins could play an imperative role towards the identification of molecular mechanism leading to the pathology of schizophrenia ,which still remains to be explored. Further characterization will elucidate the functional mechanisms underlying defective metabolic pathways ,which will help in future therapeutic developments in the treatment of schizophrenia.

PP01.220

Pharmacological and Functional Characterization of the Endogenous LPA Receptors in U87-MG Cells

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Abstract: Lysophosphatidic acid (LPA) is a bioactive lipid that participates not only in cell metabolism, but also as an autacoid and local hormone. LPA is involved in a large number of physiological processes, modulating the function of many organs and systems. As a lipid mediator, it takes part in embryonic development and it is also involved in the pathogenesis of many diseases. LPA modulates migration, proliferation, survival and other processes. LPA actions are mainly exerted through a family of six G protein-coupled receptors that are designated LPA_{1-6}. In this work, we studied the LPA receptors that are endogenously expressed in the cell line U87-MG, a human glioblastoma and their signaling. Our results indicate that LPA was able to increase intracellular calcium concentration ($[Ca^{2+}]_i$) and ERK1/2 phosphorylation. OMPT, a selective LPA_1 agonist also induced these effects which were inhibited by selective antagonist for $LPA_{1/3}$ (Ki16425) and LPA_1 (AM095) receptors. The data suggest the possibility that $LPA_{1/3}$ receptors are endogenously expressed in these cells. On the other hand, pharmacological activation of protein kinase C by phorbol myristate acetate (PMA) resulted in a decrease of $_i[Ca^{2+}]$ when cells were challenged with LPA; this effect was reverted by specific PKC-inhibitors, suggesting that these receptors are modulating by conventional PKC isoforms. Interestingly, PMA and AM095 induced a decrease in the basal levels of $[Ca^{2+}]_i$ in a dose-response manner, which suggest that LPA_1 receptors may exhibit endogenous activity. Finally, cell size was decreased by LPA and OMPT and this effect was blocked by Ki16425 and AM095.

PP01.221

House Dust Mite Allergen Suppresses Neutrophil Apoptosis by Cytokine Release via PAR2 in Normal and Allergic Lymphocytes

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Abstract: House dust mite (HDM) is an essential allergen in allergic diseases such as allergic rhinitis and asthma. The pathogenic mechanism of allergy is associated with cytokine release of lymphocytes and constitutive apoptosis of neutrophils. In this study, we examined whether HDM induces cytokine release of lymphocytes and if the secretion of cytokines is involved in modulation of neutrophil apoptosis. In normal and allergic subjects, extract of *Dermatophagoides pteronissinus* (DP) increased IL-6, IL-8, MCP-1, and GM-CSF secretion in a time-dependent manner. This secretion was suppressed by PAR2i, an inhibitor of PAR2, in a dose-dependent manner, as well as by LY294002, an inhibitor of PI3K, AKTi, an inhibitor of Akt, PD98059, an inhibitor of ERK, and BAY-11-7085, and an inhibitor of NF-κB. ERK activation was suppressed by PAR2i, LY294002 and AKTi, and NF-κB activation was blocked by PAR2i, LY294002, AKTi, and PD98059. Supernatants collected from normal and allergic neutrophils after DP treatment inhibited the apoptosis of normal and allergic neutrophils through suppression of caspase 9 and caspase 3 cleavage. In summary, DP induces the release of cytokines through the PAR2/PI3K/Akt/ERK/NF-κB pathway, which has anti-apoptotic effects on neutrophils of normal and allergic subjects. These results will facilitate elucidation of the pathogenic mechanism of allergic diseases.

PP01.222

Hypoxia Disrupts, While Tongxinluo Protects the Vascular Endothelium by Inducing TJ Protein Expression Mediated by KLF4

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Abstract: Vascular endothelium forms a continuous inner lining of the blood vessels, and permits the movement of molecules between circulating blood and interstitial space. The hypoxia can cause abnormal expression of tight junction proteins, leading to disruption of tight junctions (TJs) and increasing permeability of the endothelial barrier. Tongxinluo (TXL), a traditional Chinese medicine that is extracted, concentrated and standardized from a mixture of 12 medicinal constituents, can improve endothelial cell function and protect the brain against blood-brain barrier disruption. However, it remains unclear whether there is a direct relationship between protective effect of TXL on endothelial functions and TXL-induced tight junction protein expression. The aim of present study was to investigate the mechanism of TXL actions whereby TXL protects against hypoxia-induced tight junction disruption. We found that hypoxia disrupted, while TXL treatment protected the TJs in the microvasculature of the mouse brain, and that TXL promoted the expressions of TJ proteins VE-cadherin, β-catenin and ZO-1 in human cardiac microvascular endothelial cells (HCMECs) under hypoxia conditions. Mechanistic studies suggested that upregulation of TJ protein expression is attributable to KLF4 phosphorylation at sites Ser444 and Ser415 induced by TXL. Furthermore, our study shows that Akt signaling plays a key role in TXL-induced KLF4 phosphorylation. In conclusion, the results of our study reveal a novel mechanism whereby TXL protects against hypoxia-induced tight junction disruption through promoting KLF4 phosphorylation and inducing TJ protein expression.

PP01.223

HSD17B4 Is a Link Between Estradiol and Inflammation in Hepatocarcinogenesis

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Abstract: Hepatocellular carcinoma (HCC) arises in a setting of chronic inflammation induced by inflammatory cytokines, such as nuclear factor-kappa B (NF-κB), and can be attenuated by estradiol (E2) in vitro and in vivo experiments. 17β-hydroxysteroid dehydrogenase 4 (HSD17B4) catalyzes the conversion of E2 to estrone and its promoter sequence contains putative NF-κB elements. However, the role of HSD17B4 in HCC is currently unknown. In this study, we investigated the function of HSD17B4 during HCC progression and related mechanism using clinic tumor paraffin sections, animal HCC model, xenograft model, and cell line. Our results indicated that HSD17B4 is highly expressed in liver tumor tissues both from HCC patients and Diethylnitrosamine-induced HCC model rats, and tumor necrosis factor alpha (TNF-α)-stimulated HCC cell line HepG2. HSD17B4 over-expression in HepG2 cells significantly promoted its E2-inactivating activity and the cell proliferation in vitro, and the growth of xenograft and the decrease in serum E2 levels of nude mice bearing HepG2 cells. NF-κB was highly co-localized with the NF-κB - element of HSD17B4 in liver tumor tissues from HCC patients. We demonstrated that the human HSD17B4 is an NF-κB target gene, a proliferation-promoting protein and a link between estradiol and inflammation. The mechanism was proposed: activated NF-κB stimulates the expression of HSD17B4 and interleukin 6 by binding to NF-κB-element, which, in turn, promotes hepatoma cell proliferation via inactivating E2 and up-regulating cyclin D1 and proliferating cell nuclear antigen. The inhibition of HSD17B4 expression may be a novel therapeutic target to ameliorate liver cancer cell dysfunction.

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