5th Annual University of Kentucky Society of Postdoctoral Scholars Regional Research Symposium

Abstract Book

May 31, 2019

Oral Presentation Abstracts

10:10 AM – Yohan Choi, Ph.D. – College of Medicine, University of Kentucky

FOS/AP-1 Transcription Factor: a Critical Regulator in Human Ovulatory Follicles

FOS is a subunit of activator protein-1 (AP-1) transcription factor and functions by forming heterodimeric complexes with one of JUN family proteins (JUN, JUNB, and JUND). FOS/AP-1 is involved in various physiological events by directly regulating the transcription of numerous genes. A recent study showed that Fos null mice failed to ovulate even when given exogenous gonadotropins, indicating that the FOS/AP-1 plays an essential role in normal ovarian function. Previously we have shown that the expression of FOS and JUN family proteins increases in ovulatory follicles of humans. However, little is known about the regulatory mechanisms by which the LH surge upregulates FOS and JUN family members and the specific function of the FOS/AP-1 in human ovulatory follicles. To determine the regulatory mechanism and function of FOS/AP-1, human granulosa/lutein cells (hGLC) from IVF patients were cultured for 6 days to recover their LH/hCG responsiveness and then treated with various reagents for selected time points. First, we found that hCG increased FOS expression (mRNA and protein) in a biphasic manner; the expression increased within 1 h but decreased by 6 h after hCG treatment. The second rise was observed at 12 h, but then the levels returned to control levels by 24 h. Unlike FOS, hCG transiently increased JUNB mRNA levels only at 1 h after hCG but had no effect on the levels of mRNA for JUN and JUND at any time points assessed. In contrast, Western blot analysis revealed increases in proteins for all Jun family members in hCG-treated cells although the temporal patterns are slightly different among them. Next, to determine which JUN family proteins are partnered with FOS, the co-immunoprecipitation analysis was performed with hGLC cultured with or without hCG for 3 or 12 h. The data showed that FOS interacts with all JUN proteins in hCG-treated cells at both time points. To further elucidate which signaling pathways are involved in rapid induction of FOS expression, several potential signaling pathways and their involvement in FOS expression were investigated using hGLC cultured with or without hCG. hCG increased the levels of both phosphorylated CREB (pCREB) and pERK1/2 within 0.5h, and their levels were sustained by 1 and 3h, respectively. Meanwhile, pAKT and p38MAPK levels were not affected by hCG. Inhibitors for PKA (H89) and ERK (SCH772984) signaling pathways completely blocked hCG-induced increases in the levels of FOS and JUN protein at 1 h. Finally, to unravel the role of FOS/AP-1 complexes, hGLC were treated with or without hCG ± T- 5224 (FOS inhibitor). T-5224 partially inhibited hCG-induced increases in the levels of PGR mRNA and protein, but did not affect AREG, EREG, and ID2 in hGLC collected at 3 and 6h after hCG treatment. In addition to PGR, T-5224 reduced hCG-induced increases in prostaglandin E2 levels and the hCG-stimulated cell metabolic activity without affecting progesterone levels at 24h after hCG stimulation. Consistent with these findings, T-5224 inhibited hCG-induced increases in the expression of prostaglandin E synthase (PTGES) and prostaglandin transporters (SLCO2A1 and ABCC4) at 12h after hCG treatment. ChIP analyses confirmed the direct binding of FOS on chromatin fragments containing FOS/AP-1 binding sites in the promoter region of PGR, PTGES, SLCO2A1, and ABCC4 genes. Collectively, these data revealed the hCG-induced temporally regulated increases in the expression of FOS and JUN family members and the involvement of hCG-triggered PKA and MAPK signaling pathways in the immediate early induction of FOS.

Fuctional studies indicated that FOS/AP-1 acts a key mediator of ovulation by regulating the expression of key ovulatory genes, production/transport of prostaglandins, and activity of granulosa cell metabolism in human periovulatory follicles. This research was supported by P01HD71875 and R03HD088866.

10:20 AM – Belal Muhammad, Ph.D. – Cincinnati Children's Hospital Medical Center

New Therapeutic Strategies for Cancers with Transcription Elongation Defects (TEdeff)

The concept of Transcription Elongation Deficient (TEdeff) phenotype has been recently described as a previously unknown epigenetic mechanism in a wide range of human cancers (Modur et al, 2018). Tumors with TEdeff phenotype which exist in more than 20% of all human cancers are characterized by defects in RNA Polymerase II elongation along with extensive overexpression of the cytoplasmic protein homeostasis machinery, including several components of autophagy and proteasome degradation pathways. Here, we show that TEdeff cancer cell lines are suffering from a profound accumulation of protein aggregates that associated with autophagic influx for clearance. Importantly, the TEdeff cells, but not their parental control TEprof [cells with proficient transcription elongation], are highly sensitive to the autophagy but not proteasome inhibitors. Mechanistically, we found that blocking the autophagy pathway maximizes the proteotoxic stress leading to a strong synthetic lethality in TEdeff cells. This treatment strategy has major therapeutic implications especially as TEdeff is a wide-spread global epigenetic phenomenon across all tumor types.

10:30 AM –James Castle, Ph.D. – College of Medicine, University of Kentucky

Estimating Breast Tissue-Specific Epigenetic Age Using Next-Generation Methylation Sequencing Data

Epigenetic age captures both the genetic and environmental influences across time on cellular functions and is an indicator of biological aging. Epigenetic age may deviate from chronological age substantially in individuals. Epigenetic age is also tissue-specific. Emerging evidence suggests that female breast tissue ages faster than other parts of the body according to epigenetic age estimation using the "Horvath Clock" model. The Horvath method is based on the DNA methylation of 353 CpG loci on the outdated Illumina microarray platforms. The increasing availability of next-generation sequencing data calls for method development that uses DNA methylation sequencing data to estimate tissue-specific epigenetic age. We developed a new method to estimate breast tissue-specific epigenetic aging using next-generation methylation sequencing data and assessed the difference between epigenetic and chronological ages, known as epigenetic age acceleration (EAA), in different breast tissue types. The Illumina TruSeq Methyl Capture EPIC Sequencing library was used to obtain DNA methylation profiles of 3,348,777 CpG sites in 109 tumor, 45 matched adjacent normal, and 462 normal breast tissue samples. A total of 3,316,665 CpG sites remained after quality control. Following the Horvath approach, we used an elastic net penalized regression model to regress chronological age on CpG sites in normal breast tissue and defined a new set of 280 clock CpGs specific to breast tissue with randomly divided training (n = 370) and testing (n = 92) data sets. We estimated breast tissue-specific epigenetic age and EAA in tumor, adjacent, and normal tissue. We found that breast tissue-specific epigenetic age was positively correlated with chronological age (r=0.89; P=4.1E-32). Neither normal nor adjacent normal breast tissue showed a significant EAA. However, tumor breast tissue had a significant and increased EAA (median = 9.0 years; P=8.2X10-10). While triple-negative breast tumors showed no significant EAA, hormone receptive-positive and Her2-

positive breast tumors had a significant and increased EAA (median=10.1 and 12.6 years; P=0.02 and 2.3E-5, respectively). In addition, EAA was assessed for tumors of varying stages and Nottingham grades. Tumors of grades 6, 7, and 8 were found to have a significant and increased EAA (median = 22.1, 11.8, and 4.2 years; P=2.1E-4, 0.03, and 0.03, respectively); stage II tumor tissues exhibited a similar EAA behavior (median = 19.1 years; P=0.007). Race association with epigenetic age acceleration was explored in cancer subtype groups and a significant association was observed for Caucasian vs. African American women in Her2+ breast cancer samples (beta=19.0 years; p=0.038). Further research is needed to determine whether epigenetic age acceleration in normal breast tissue is predictive of breast cancer risk and how breast cancer risk factors influence the rate of acceleration

2:00 PM – Xingjiang Mu, Ph.D. – College of Medicine, University of Cincinnati

Sectm1a positively regulates tissue-resident macrophage self-renewal capacity during endotoxemia by boosting T effector cells

Introduction: Endotoxin-induced organ failure and mortality are largely ascribed to an inappropriate activation of innate immune cells i.e., macrophages. Currently, most studies have focused on the differentiation (M1 or M2) of recruited-monocytes during endotoxemia, however, less is known about tissue-resident macrophages with self-renewal and homeostasis-maintaining capacity. Previously, we demonstrated that mouse Sectm1a, a transmembrane and secreted protein highly expressed in cells of the myeloid lineage and epithelia, could attenuate endotoxin-induced decrease of proliferation in splenic tissue-resident macrophages. Nevertheless, it remains unknown whether Sectm1a preserves the self-renewal capacity of tissue-resident macrophages in other organs such as heart and lung during endotoxemia. Methods: LPS was administered into Sectm1a-knockout (KO) and wild-type (WT) mice by intraperitoneal (i.p.) injection (10 μg/g). At different time points, heart and lung samples were collected to determine the local proliferation of resident macrophages through flow cytometry analysis. In addition, splenic T cells and macrophages were isolated, co-cultured and stimulated by LPS with or without recombinant Sectm1a protein (rSectm1a) treatment. Results: For cardiac tissue-resident macrophages (CD45.2+ly-6G-F4/80+CD64+CD11b+), we observed a significant decrease (~15%) of Ki-67+, a key marker of proliferation, at 18-h post-LPS injection (n=8). By contrast, inflammatory ly-6Chigh CCR2+monocyte numbers were dramatically increased from 2% to 24% of total Mφ/Mo in mouse hearts following LPS administration. Interestingly, loss of Sectm1a further accelerated the reduction of Ki-67+ resident macrophages to a great degree (~30%), which was accompanied with more infiltration of inflammatory monocytes (36% of total Mφ/Mo) in hearts after LPS exposure, compared to WT controls (n=8, $P < 0.05$). At the same time, similar results were found in the lung of LPS-treated mice. Given that Sectm1a has been shown to promote T-cell proliferation, we next determined whether T-cells contributed Sectm1a-elicted effects on macrophages by co-culturing macrophages with splenic naive T cells in the present or absence of rSectm1a following LPS exposure. We found that rSectm1a treatment not only stimulated naive T cell proliferation but also promoted the differentiation of naive T cells into Th1 and Th2 effector cells, and increased IL-4 release, which, in turn, significantly rescued the proliferation of resident macrophages (n=6, $P < 0.01$) under LPS challenge. In addition, our in vivo experiments demonstrated that endotoxin administrations furtherly reduced both proliferation and polarization of T cells in spleens of KO mice, compared to those in WT mice (n=6, $P < 0.01$), suggesting Sectm1a's important roles in boosting naive T cell during the inflammatory response. Conclusion: Sectm1a plays a critical role in maintaining self-renewal capacity of tissue resident macrophages

during sepsis through boosting T effector cell polarization. Enhancing the Sectm1a level may serve as a promising therapeutic approach for sepsis.

2:10 PM – Patrick Van Hoose, Ph.D. – College of Medicine, University of Kentucky

Lipid Phosphate Phosphatase 3 in Smooth Muscle Cells Regulates Dissecting Abdominal Aortic Aneurysm Formation

Lipid phosphate phosphatase 3 (LPP3), encoded by PLPP3, is a cell surface enzyme that regulates lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) availability and signaling. Genome wide association studies in humans identified heritable single nucleotide polymorphisms (SNPs) in the final intron of PLPP3 that independently predicted coronary artery disease (odds ratio, 1.17; P=3.81×10–19). The risk allele reduces gene expression by disrupting binding of CCAAT enhancer binding protein beta (CEBPβ). These observations have focused attention on the role of LPP3 in atherosclerotic vascular disease. Global loss of Plpp3 or smooth muscle cell (SMC) specific inactivation of Plpp3 promotes the development of experimental atherosclerosis or intimal hyperplasia in mice. Together, these results indicate that LPP3, perhaps in part due to its ability to degrade LPA and thereby limit LPA signaling functions to mitigate SMC proliferation and vascular inflammation. At present, little is understood about what regulates Plpp3 expression during vascular disease. We observed 72hr angiotensin II (AngII) treatment is a strong activator of PLPP3 expression in coronary artery human SMCs (caHSMCs), through pathways that involve NF-κB, as parthenolide, an inhibitor of IκBα degradation, blocked ATII induced PLPP3 expression. To understand the functional consequences of AngII promoted LPP3 expression, we infused AngII into mice with SMC specific inactivation of Plpp3 (SM22-Δ) or littermate controls (fl/fl). SM22-Δ mice displayed a significant reduction in abdominal aortic diameter and smooth muscle alpha actin (Acta2) gene expression compared to fl/fl mice. Histone 3 lysine 4 dimethylation (H3K4dime) within the myosin heavy chain 11 (Myh11) gene, a stable epigenetic marker of SMC lineage, was not significantly changed. These results indicate that smooth muscle cells from SM22-Δ mice may undergo phenotypic modulation and in support of this hypothesis we observed increased vimentin, a fibroblast marker, expression within the SMC medial layer of abdominal aortas in SM22-Δ mice. Overall these results demonstrate a novel pathway for regulation of LPP3 during vascular disease involving a NF-κB dependent pathway and in the absence of LPP3, SMCs assume a more dedifferentiated fibroblast like phenotype that is associated with protection against abdominal aortic aneurysm.

2:20 PM – Syed Z. Islam, Ph.D. – Oak Ridge National Laboratory

Recovery and Recycling of Rare Earth Elements from Electronic Waste

In recent years, rare earth elements (REEs) such as Nd, Pr, and Dy have drawn tremendous attention due to their substantially increasing use in the form of permanent magnets in hybrid and electric vehicles, wind turbines, mobile phones, tablets, personal computers, and a wide range of devices with electric motors. To provide a domestic source for REEs, we have developed a supported membrane solvent extraction (MSX) process for the recovery of REEs from scrap permanent magnets. The separation results show that the REE recovery of >97% with purity of >99.5 wt.% can be achieved from a wide range of high-volume scrap magnet feedstocks. The results demonstrated that MSX is a robust, scalable, economically viable and sustainable, and

environmentally friendly process for the recovery of REEs from various types of scrap magnet sources.

2:30 PM – Muhammed Fethullah Simsek, Ph.D. – Cincinnati Children's Hospital Medical Center

Multicellular Signaling Network Provides Time and Position Information for Robust Patterning

Cell to cell signaling provides instructive information for numerous decision-making processes during patterning of developing embryos. A striking example of precise, scalable and tissue-level patterning is segmentation of somites from the presomitic mesoderm (PSM). Somites, in bilateral pairs, segment from the PSM sequentially with species-specific time intervals until completing a species-specific total somite count, regardless of embryo to embryo variabilities. For this robust and repetitive patterning to happen, Notch signaling, via traveling expression waves of a molecular clock network, provides time information. On the other hand, the fibroblast growth factor (FGF), Wnt, and retinoic acid (RA) pathways establish spatial gradients in the tissue. Despite decades of studies proposing roles for all three pathways, the dynamic feature of these gradients that encodes instructive position information determining segment sizes remained elusive. Here, we developed a tail explant system in zebrafish allowing untangling the molecular signaling dynamics from axis elongation dynamics. From a systems biology perspective, we integrated quantitative microscopy with computational modeling, and tested alternative models to show that the position information is encoded solely by spatial fold change (SFC) in FGF signaling. Neighboring cells measure SFC to accurately position the determination front and thus determine segment size. The SFC model successfully recapitulates results of spatiotemporal perturbation experiments on both explants and intact embryos, and it shows that Wnt signaling acts permissively upstream of FGF signaling and that RA gradient is dispensable in segmentation. A tight coupling between FGF and segmentation clock signaling lets a posteriorly regressing FGF gradient to be integrated with an anteriorly traveling clock wave to establish the determination front, i.e. segmentation of discrete somites.

2:40 PM – Ian Boggero, Ph.D. – College of Medicine, University of Kentucky

Using Sleep Parameters to Predict Pain and Disability Outcomes in an Inpatient Functional Rehabilitation Program for Children with Chronic Pain

Self-report and objective (actigraphy) measures of sleep have been shown to differentially predict pain-related outcomes, but no study has compared these measures in their ability to predict functional disability or pain coping in an inpatient rehabilitation program. The current study used data from 41 children and adolescents (Mean age = 14.8, SD = 2.74, age range = 9-18) who were in an inpatient functional rehabilitation program for chronic pain. Children were in the program between 18 and 32 days (Mean days = 20.57, SD = 4.98). Self-report sleep measures of sleepiness (Epworth Sleepiness Scale), sleep quality (Adolescent Sleep/Wake Scale), hygiene (Adolescent Sleep Hygiene Scale), and insomnia (Pediatric Insomnia Severity Index) were collected at program admission. Actigraphy sleep continuity measures (sleep duration, sleep efficiency, total sleep time, and wake after sleep onset) were collected by averaging data from an actigraphy watch worn over the first 7 nights in the program. Functional disability (Functional Disability Inventory) and pain coping (Pain Coping Questionnaire) were collected at admission and discharge and a difference score was computed. Results revealed significant improvement in disability ($M_{admission}$= 30.96, $M_{discharge}$= 12.30, $t(38) = 9.93$, $p < .001$) and pain coping over the course of the program ($M_{admission}$= 8.61, $M_{discharge}$= 10.87, $t(38)=-5.47$, $p<.001$). Self-report sleep measures were more strongly associated with improvements in functional disability

and pain coping than actigraphy measures (average =.28 vs =.09 for functional disability; Average =.24 vs. .20 for pain coping). The strongest associations were with poor sleep hygiene at baseline predicting improvements in disability (=.47, p=.035). This is the first study to compare self-report and actigraphy sleep measures for predicting disability or pain coping in an inpatient rehabilitation program for children with chronic pain, and highlights the value of assessing sleep subjectively.

2:50 PM – Mereena George Ushakumary, Ph.D. – Cincinnati Children's Hospital Medical Center

Defining the role of activated fibroblasts in cystic fibrosis

Cystic Fibrosis (CF) remains one of the most common life-threatening genetic disorder due to mutations of the CF Transmembrane Conductance Regulator (CFTR) gene. Mortality of patients with CF is mainly due to chronic lung infection, inflammation and fibrosis. End-stage CF lung disease with pulmonary fibrosis and increased deposition of collagen has been demonstrated in distal airways of children. Though small molecule treatments correcting or potentiating CFTR shown clinical benefits, they are limited only to a few CFTR mutations and are inadequate to address challenges associated with clinical heterogeneity of the disease such as significant loss of lung function and lung parenchyma in older CF patients. Although much of the knowledge regarding CF seems to have been gained by studying airway epithelial cells and blood cells, the role of pulmonary fibroblasts in mediating the CFTR mutation-induced changes is largely unidentified. The present study used human CF lung tissue which carries F508 deletion mutation, which is estimated to be the cause of ~ 67% of CF and explored a role for PDGFRα+ve fibroblasts in mediating CFTR-mutation induced remodeling in the lung. Massive fibrosis in CF lung was evidenced by H&E staining. Second Harmonics Generation (SHG) and immunofluorescence showed an increase in collagen and SMA, respectively. An increase in fibroblast markers such as vimentin and PDGFRα was also observed in CF lung along with an increased PDGFA ligand. Further, qPCR analysis showed an increase in Wnt5a and PDGFRα gene expression levels in magnetic sorted CD326- cells, showing that there is altered PDGFRα/Wnt5a signaling occur in non-epithelial cells. RNA seq data from our lab suggests a role for Wnt5a in mediating a matrix fibroblast phenotype in the lung. Therefore, it is possible that a PDGFRα/Wnt5a-driven pathological remodeling response occurs in the PDGFRα+ve fibroblasts in CF lung. The findings identify a potential role for activated fibroblasts in CF which has significant implications in the pathological remodeling of CF lung.

3:20 PM – Shailaja Hegde, Ph.D. – Cincinnati Children's Hospital Medical Center

Leukemic Initiating/Propagating Cell Basal Polarity Complex Scribble is required for leukemogenesis through the hypoxia inducing factor-1a.

Despite the introduction of tyrosine kinase inhibitor therapies, the prognosis for BCR-ABL (+) acute lymphoblastic leukemia remains poor. In the present study, we show the role of the Scribble protein in both lymphoid and myeloid leukemogenesis. The polarity protein Scribble is a member of the basal polarity complex, which is down-regulated in many cancers, suggesting a possible tumor suppressor role, especially in so-called cancer initiating cells. Its effect and mechanisms of activity in leukemic cell fate along with its potential activity on leukemic initiating cells have only been recently started to elucidated. Using interferon-responsive inducible (mx1-Cre) Scribble-deficient mice, we have characterized the role of Scribble in both retroviral transduction,

transplantation animal models and binary, inducible stem cell initiated (Scl-tTA/TRE-BCR-ABL) serial propagation models of BCR-ABL induced leukemia. We found that Scribble expression is upregulated at both transcriptional and translational levels in p210- or p190-BCR-ABL induced leukemic progenitors. In vitro, leukemic colony formation was impaired in Scribble deficient leukemic progenitors (~48% reduction; p≤ 0.05, compared to Wt leukemic progenitors) demonstrating that Scribble is important for leukemogenesis. The deficiency of Scribble abrogates myeloproliferation and leukemic myeloid progenitor self-renewal. In vivo, the deletion of Scribble abrogates the development of myeloproliferative disease. p210-BCR-ABL (median survival: 70 vs 47 days in Scribble deficient and Wt chimeric mice, respectively; p≤0.05); and significantly impairs B-cell lymphoid leukemogenesis induced by p190-BCR-ABL (median survival: 80 vs 60 days for Scribble deficient and Wt chimeric animals, respectively); Furthermore, Scribble deficiency in leukemic progenitors increases the activation of the AMPK/mTORC1 signaling pathway and the protein expression and transcriptional activity of its downstream effector hypoxia-inducing factor-1α (HIF-1α). HIF-1α silencing by constitutive shRNA expression or inducible deletion in Scribble deleted B-lymphoid leukemic cells restored leukemic progenitor (p≤0.01) and B-lymphoid leukemogenesis in vivo (median survival of 62 days; (p≤0.05 compared with Scribble deficient chimeric animals). In addition, double deficiency of Scribble and HIF-1α restored AMPK/mTORC 1 signaling to Wt leukemic levels. This data indicates that Scribble is a negative regulator of HIF-1α expression and activity, and the downregulation of HIF-1α expression and activity is necessary to restore leukemia initiation and propagation. Altogether, our data indicates that Scribble is a positive regulator of oncogenesis in leukemic myeloid and lymphoid progenitors, in vitro and in vivo, through HIF-1α activities.

3:30 PM – Velmurugan Gopal Viswanathan, Ph.D. – College of Medicine, University of Kentucky

Amylin dyshomeostasis impairs Aβ clearance and blood-brain barrier function by miRNA mediated LRP1 and ZO-1 downregulation

Amylin dyshomeostasis is a comorbidity in human type-2 diabetes (T2D). Amylin interacts with amyloid beta (Aβ) accumulation in Alzheimer's disease (AD). However, the molecular mechanism is not known. This study explores the mechanism of amylin dyshomeostasis mediated Aβ accumulation in the brain. Amylin significantly downregulates low-density lipoprotein receptor-related protein-1 (LRP1) and tight junction protein (ZO-1) in primary rat brain microvascular endothelial cells (RBMVEC) that regulate brain Aβ dynamics. Amylin upregulates miR-103 and miR-107 expression while transfection of miR-103 and miR-107 downregulates LRP1 and ZO-1 expression in RBMVEC by binding at 3′ UTR region. In functional in-vitro blood-brain barrier (BBB) studies, amylin reduces trans-endothelial electrical resistance (TEER) and decreases Aβ clearance from the brain side to blood side. Consistently, LRP1 expression in brain capillaries is downregulated and Aβ accumulation is enhanced in human amylin overexpressing rat model. In conclusion, amylin dyshomeostasis in T2D exacerbates Aβ accumulation in brain through impaired LRP1 and ZO-1 expression.

3:40 PM – Megan Johnstone Ph.D. – Cincinnati Children's Hospital Medical Center

Investigating DEK as a prognostic marker for secondary breast cancer in female survivors of pediatric Hodgkin's Lymphoma

Approximately 35% of young females with HL treated with chest irradiation will develop secondary breast cancer (sBC) by age 50. The majority of these patients are diagnosed a more advanced,

bilateral, aggressive disease with nearly 100% mortality. Radiotherapy is a known malignant initiator by inducing different types of DNA damage. DNA repair mechanisms are linked to cell cycle phase. The expression DNA repair proteins during radiotherapy are likely to correlate to risk of sBC and have the potential to be prognostic and/or predictive markers. DEK, a DNA repair protein, is required for high fidelity DNA repair responses to radiation and constitutive expression stimulates cell cycle progression. Exposure to radiation in a quiescent cell cycle state results in increased DNA damage, low fidelity repair, and ultimately increased genetic mutations. Conversely, in primary breast cancers, constitutive activation of DEK promotes an oncogenic phenotype and is an independent marker of poor prognosis. Breast development is driven by asymmetric mammary stem cell division. Mammary stem cells cycle through quiescence and active proliferation with hormonal and molecular signaling throughout puberty. DEK plasma levels correlate with some clinical and pathological variables in patients with primary breast cancer. Taken together, DEK is an important regulator of DNA repair and pro-tumorigenic signaling particularly in stem cells and may be a predictive and/or prognostic biomarker for sBC after radiotherapy.

3:50 PM – Byeongjae Ben Chun, Ph.D. – College of Arts and Sciences, University of Kentucky

Computational Modeling of Microglial Physiology and Pathophysiology

Microglia are the immunocompetent cells residing in the central nervous system (CNS), whose roles are known to be surveillance, scavenging and repairing/regenerating damaged or dead cells in tissue due to the insults or degenerative diseases such as Alzheimer's disease. Based on the aforementioned immunogenic roles of microglia in the CNS, numerous studies have discovered and claimed that activated microglial cells are not only "preventing" the damage but also "deteriorating" the conditions by harming healthy surroundings. Our current project utilizes multiscale simulation techniques, each of which suit to specific time or length scales, to identify the most governing pathway(s) that may cause significant dysfunction of microglia observed in tissue sample of patients. As start, the research specifically focuses on crucial microglial activation pathways mediated by the most common extracellular factors such as adenosine triphosphate (ATP) and lipopolysaccharide (LPS) that represent damage-associated molecular pattern (DAMP) and pathogen-associated molecular pattern (PAMP), respectively. Besides, we also explore pro- and anti-inflammatory cytokines that are determined to be key players for phenotype polarization via either autocrine or paracrine signaling pathways. Via this presentation, we introduce not only the current progress in each computation scale, but also the philosophy of multiscale computational approach along with the ultimate goal. For instance, molecular dynamics (MD) simulation is utilized to determine the thermodynamics involved with molecule-protein or protein-protein interaction in order to robustly estimate the kinetics of interactions mediated by various factors including the aforementioned stimuli and cytokines that are released from surroundings. Based on these findings, dynamics of downstream signaling is reproduced using deterministic/stochastic differential equations, the outcomes of which will explain the mechanisms of such dramatic change in intra/extracellular environment of microglia. Finally, these changes are correlated to the morphological transformation of cells and the distinct difference in mobility among resting and activated microglia for demonstrating the further prediction in response to physiological and pathological conditions.

4:00 PM – **Maria Kraemer, Ph.D.** – College of Medicine, University of Kentucky

Dietary Regulation of Lipoprotein Associated Bioactive Lysophospholipid Mediators of Atherosclerosis

Lysophosphatidic acids (LPAs) are a family of bioactive lipids. LPA and genes involved in LPA metabolism and signaling have been linked to atherosclerosis in preclinical models and are associated with heritable cardiovascular disease risk in humans. LPA accumulates in atheromas suggesting that LPA associates with atherogenic low density lipoproteins. LPA is present in LDL, but the source, regulation and biological activity of LDL-associated LPA is unknown. We used mass spectrometry to examine how diet and genetically induced hyperlipidemia affects the levels and distribution of LPA in mouse plasma. In wildtype mice fed western diet, LPA was mostly associated with albumin; however, we observed a 3-4 fold increase in LPA associated with LDL and VLDL in hyperlipidemic mice (LDLr-/-, ApoE-/-, and PCSK9 treated C57Bl6) fed a western diet. These increases in LDL-LPA were not observed in mice with adipocyte-specific genetic deficiency of autotaxin, a lysophospholipase D known to generate LPA. We also found that autotaxin directly acts on LDL associated lipids to generate LPA. Our data indicates that LDL-LPA may be a link between diet and cardiovascular disease risk.

4:10 PM – **Hanane Boukarabila, Ph.D.** – Cincinnati Children's Hospital Medical Center

Towards understanding & uncovering new key players in T cell development upon aging

Background: The decline of the immune system, which is referred to as aging-associated immune remodeling (AAIR), that occurs upon aging is due to many factors acting in concert. AAIR leads to an impaired ability to respond to vaccination and combat infections and therefore impacts negatively on the quality of life of the elderly. In contrast to other blood lineages, which develop primarily in the bone marrow (BM), the T-cell lineage develops in the thymus by continuous replenishment of thymic seeding progenitors (TSPs) from the BM. Several studies have linked AAIR to thymic involution. However, there is novel and mounting evidence that also aging of hematopoietic stem cells (HSCs) and lymphoid-primed multipotent progenitors (LMPPs) are immune system intrinsic players in AAIR. Very little, though, is known on mechanisms by which aging of HSCs and LMPPs contribute to AAIR. Hypothesis: A central aim in understanding the AAIR is identifying how aged HSCs and LMPPs could possibly drive this phenomenon. Few possibilities could individually or in combination explain such a defect in an intrinsic fashion: aged HSCs and LMPPs could give rise to a defective TSP population associated with either: 1- size reduction, and/or, 2- lineage potential bias, and/or, 3- homing defect, already in the BM, that would fail to engage in normal T-cell differentiation. Results: Our findings demonstrate that both aged HSCs and aged LMPPs retain the T-lineage potential in ex-vivo at the single cell level and in vivo upon transplantation assays. However, the detailed analysis of transplanted young recipients by aged HSCs shows significant decrease in the pool size of LMPPs in the BM and early thymic progenitors (ETPs) in the thymus, confirming that the old BM and thymic microenvironment could not be the sole reason behind this defect. Importantly, we were able to show that aged LMPPs are associated with a dramatic disadvantage in the development of early thymic T-cell populations as well as generating mature T-cell populations when intravenously transplanted along young LMPPs into young recipients in competitive settings. Whereas, similar competitive transplantation assays when injected intra-thymically led mainly to a dramatic disadvantage towards generating more matures T-cell stages, suggesting that aged LMPPs are associated with thymic developmental defect and a potential homing defect that occurs with age. Conclusion: AAIR is a

consequence of multiple immune parameters at play and we present here new data demonstrating a role for aging of LMPPs and changes in early thymic differentiation events in driving AAIR. Understanding how aged HSCs and LMPPs could possibly drive this AAIR phenomenon at the cellular and the molecular level is of crucial importance for developing new therapies to attenuate AAIR.

4:20 PM – Nirmal Verma, Ph.D. – College of Medicine, University of Kentucky

Prediabetic Hypersecretion of Amylin Alters Oxygen Sensing and Accelerates Aging

Capillary function and oxygen-carrying capacity of red blood cells (RBCs) decline in type-2 diabetes exacerbating the risk of hypoxia and organ malfunction. Amylin is a β-cell hormone that forms pancreatic amyloid in patients with type-2 diabetes and its blood level is elevated in prediabetes. Given the amyloidogenicity of human amylin, we hypothesized that hyperamylinemia increases the risk of hypoxia by provoking microcirculatory disturbances. Using rats with pancreatic overexpression of human amylin (HIP rats) and transfusion with RBCs from diabetic HIP rats into normal rats, we show that the transition from prediabetes to diabetes is associated with amylin deposition in capillaries and RBCs, which increases RBC to endothelial cell adherence, decreases RBC hemoglobin and activates hypoxia-inducible factors in endothelial cells leading to arginase-nitric oxide dysregulation. Prediabetes-induced amylin dyshomeostasis accelerates aging in HIP rats with multi-organ impairments and increased mortality. Upregulation of epoxyeicosatrienoic acids, which are lipid mediators formed by endothelial cells, mitigates amylin deposition in capillaries and hypoxia. In humans, amylin deposition in RBCs increases with aging in association with type-2 diabetes, heart failure, cancer and stroke. Thus, prediabetes-induced amylin dyshomeostasis impairs capillary function and oxygen-carrying capacity of RBCs; amylin-loaded RBCs can initiate pathological processes that are involved in pathological aging.

Poster Abstracts

1. **Kirtley Amos,** College of Food, Agriculture and Environment, University of Kentucky

In Pursuit of Cellulose Biosynthesis Inhibitors: A Bio-active Synthetic Compound Screen

Chemical genetics investigates the use of bioactive small molecules, as opposed to classical genetics, to disrupt target protein function. Several advantages include circumventing lethal consequences of genetic lesions in required genes or redundancy of gene families by having a common target mechanism across a gene clade. In the past 20 years, the field of plant biology has made use of chemical genetic approaches improving our understanding of cell wall biosynthesis, the cytoskeleton, hormone biosynthesis and signaling, gravitropism, pathogenesis, purine biosynthesis, and endomembrane trafficking. However, the probability of a synthetic small molecule being bioactive is low, therefore, thousands of molecules must be tested to find those of interest. Here, forward chemical genetics was applied to the problem of manipulating cellulose and lignin within plants. A 50,000 compound DIVERSet library was screened against Arabidopsis thaliana using a liquid handling robotics platform. From this screen, 3,258 compounds produced visual phenotypes and of those, 908 produced phenotypes indicative of cellulose biosynthesis alteration. Of those 908 compounds, 171 compounds cause ectopic lignification in Arabidopsis through the visual confirmation of phloroglucinol staining. Arabidopsis seeds were then exposed to each of these 171 compounds under dark growing conditions. Those compounds that inhibited hypocotyl elongation of dark-grown Arabidopsis were catalogued for further investigation. Using an ethyl methanesulfonate mutagenized population of Arabidopsis plants, we then sought resistance to our chemical ligands to link the drug to the gene. To date, putative resistance has been identified for two candidate inhibitory compounds. Resistant individuals have been outcrossed into a mapping accession called Landsberg Erecta. Growth assays point to the mutation of interest being associated with homozygous recessive resistance. 5 individual point mutations at the end of chromosome 4 have been presumably linked to the resistance to one of our compounds. T-DNA lines and structurally nearest small molecule chemical analogues will be ordered to explore further the causative point mutation within Arabidopsis as well as the functional group within the small molecule. Confocal microscopy of CesA6-GFP Arabidopsis dark-grown hypocotyl after 2 hours exposure to compounds exhibit changes in trajectory and potentially deposition into the plant membrane as seen through still images and time-lapse movies. Additional sequencing will be performed on other resistant plant lines to identify the point mutations causing resistance. Future work will be focused on in silico clustering our small molecules with known herbicides followed by using these clusters to probe all chemical space. Future study will be directed toward predicting how the small molecules of interest interact with protein targets of known herbicides.

2. **Mojtaba Bahktiari, Ph.D.,** College of Medicine, University of Kentucky

Determining the contribution of cystathionine beta synthase to lung cancer lineage fate

Background: Lung cancer is a devastating disease with high mortality even when diagnosed at an early stage. The two major pathologies of non-small cell lung cancer, adenocarcinoma (ADC) and squamous cell carcinoma (SCC) are historically treated as separate diseases, even though mounting evidence shows that epigenetic reprogramming from ADC to a more SCC fate allows lung cancers to evade therapies. Our team recently developed an isogenic lung cancer model in which lineage switching from ADC to SCC was epigenetically controlled through loss of Polycomb Repressive Complex 2 (PRC2) gene repression of squamous genes. Furthermore, we

demonstrated that both human and murine lung SCC are characterized by globally low levels of PRC2 histone mark, designated with the acronym H3K27me3. Levels of S-adenosyl methionine (SAM), the methyl donor for the complex, influence PRC2 stability and activity. Steady state metabolism showed that SAM levels were decreased significantly, and cystathionine levels were increased significantly in SCC cells relative to ADC cells. Our hypothesis is that increased activity of the enzyme cystathionine beta synthase (CBS) drives epigenetic reprogramming of lung ADC to an aggressive and therapy-resistant state through reduction of SAM pools and destabilization of PRC2, leading to expression of squamous genes. Methods: We leverage 3-dimensional organoid cultures and traditional 2-dimensional human non-small cell lung cancer cell lines to explore the contribution of CBS to lung cancer lineage fate. With both of these systems, we knocked-down CBS using lentiviral small hairpins. We have also stained a series of human lung cancer tissues for CBS and H3K27me3 expression to validate that CBS is up-regulated in squamous tumors. We have used lentiviral small hairpins targeting Cbs, and a lentivirus encoding an inducible Cbs cDNA to modulate gene and protein levels in the 3-dimensional cultures. We cloned the murine and human full length Cbs/CBS into the pLenti6 vector, and have observed robust over-expression of CBS by transient transfection.Results: Our preliminary data show that knock-down of CBS decreased cystathionine and increased homocysteine levels. We have also observed a global increase in H3K27me3 mark in shCBS cultures relative to shGFP controls. In human tumor sections, SCCs have significantly higher levels of CBS and significantly lower levels of H3K27me3 by immunohistochemistry.CBS expression may drive ADCs to a more SCC fate by causing re-expression of squamous genes normally silenced by PRC2. These changes were assessed by Western Blot, histology and gene expression analysis of matched control and CBS modulated cultures. Conclusion: Our data suggest that lung cancer lineage fate may be governed in part through expression and activity of the enzyme CBS. Our future studies include Stable Isotope Resolved Metabolomics to trace methionine usage and proteomics approaches to learn if the increase in H3K27me3 levels is due to higher activity of the PRC2 complex

3. **Anne Berres, Ph.D.**, Oak Ridge National Laboratory

Modeling Energy Use in an Urban Environment

Buildings are one of the largest energy consumers in any city's energy use, accounting for about 40% of used energy. One of the major contributing factors to energy use in buildings is presence or absence of humans. This is especially true for models that are tailored to building occupancy throughout each day. In this presentation, I will present an approach to generating a realistic model of human building occupancy throughout a typical work day that is based on the population's daily commute patterns using a transportation simulation.

4. **Cassie Chandler, Ph.D.**, College of Arts and Sciences, University of Kentucky

Modeling Ethanol and Nicotine Co-Use in Sprague Dawley Rats

Concomitant alcohol drinking and tobacco use is common, and it may be possible to develop medications to treat their co-abuse. Here, we adapted a model of alcohol and nicotine co-use to male and female Sprague Dawley rats, and assessed the ability of drug pretreatments to modulate consumption. Phase 1: 2-bottle choice between H2O and an EtOH solution. Phase 2: Nicotine self-administration under an increasing fixed-ratio (FR) schedule; 2 bottles containing H2O or 0.2% saccharin/15% EtOH were available. Phase 3: Nicotine, EtOH, varenicline, naltrexone, or vehicle were administered in a counterbalanced order; 3 maintenance sessions occurred between tests. Rats consumed relevant levels of saccharin + EtOH and nicotine at a

FR20. Pretreatment with EtOH or nicotine didn't alter EtOH consumption. Responding for nicotine was reduced by the highest dose of nicotine, EtOH, and varenicline. Naltrexone failed to elicit systematic effects. These results extend our findings in female P rats to male and female Sprague Dawley rats and further suggest that medications approved to treat alcohol or tobacco use disorders may not be effective as a monotherapy for co-use disorders.

5. Azhad U. Chowdhury, Ph.D., Oak Ridge National Laboratory

Probing the Surface Structure of Fluorinated Bottlebrush Polymers with Vibrational Sum Frequency Generation Spectroscopy and Molecular Dynamics Simulations

The molecular structure at the surface of polymer materials regulates physical and chemical properties such as reactivity, wettability, adhesion, friction, and biocompatibility. In recent years, bottlebrush polymers have gained attention in polymer research due to the ability to tune their unique architecture and functionality by tuning side chain length and composition. Bottlebrush polymers are comprised of numerous polymeric side chains densely grafted to a polymer backbone, the highly branched structure results in a steric repulsion between neighboring polymer sidechains, forcing these macromolecules to adopt a chain-extended conformation. While aspects of these polymeric structures can be characterized using standard approaches, the surface functionality and structure remain poorly understood due in part to challenges in selectively probing the interfacial layer. In this work we employ a unique combination of precision synthesis, molecular dynamics simulations, and surface specific nonlinear spectroscopy to elucidate the structure−property relationship of bottlebrush polymer surfaces. Using vibrational sum frequency generation spectroscopy (SFG), which is exquisitely sensitive to interfacial ordering and local symmetry and chemical compositions, we provide new insight into the surface structure of a recently synthesized fluorinated bottlebrush polymer based on poly(2,2,2-trifluoroethyl methacrylate). SFG measurements reveal a unique molecular structure at the surface that connects the surface composition and ordering of specific functional groups with the hydrophobicity. Changes in chain length for both bottle brush and linear analogs show a change in surface structure that is in agreement with coarse grained molecular dynamics simulations. This insight into the surface composition and structure is expected to drive new advances for future applications.

6. Swapneeta Date Ph.D., Oak Ridge National Laboratory

Insights into the Biomolecular Mechanisms of Mercury Methylation by Anaerobic Bacteria

The concentration of Hg in the biosphere has increased dramatically over the last century as a result of industrial activities. The microbial conversion of inorganic Hg to MeHg is a global public health concern due to bioaccumulation and biomagnification of MeHg in food webs. Exposure to neurotoxic MeHg through the consumption of fish represents a significant risk to human health and can result in neuropathies and developmental disorders. Anaerobic microbial communities in sediments and periphyton biofilms have been identified as sources of MeHg in aquatic systems, but the associated biomolecular mechanisms are not fully understood. In the present study, we investigate the biochemical mechanisms and kinetics of MeHg formation by HgcAB in anaerobic microorganisms. We report the kinetics of Hg methylation in cell lysates of Desulfovibrio desulfuricans ND132 at nanomolar Hg concentrations. The enzymatic Hg methylation mediated by HgcAB is highly oxygen-sensitive, irreversible, and follows Michaelis-Menten kinetics with an apparent KM of 3.2 nM and Vmax of 19.7 fmol·min-1·mg-1 total protein for the substrate Hg(II). Although the abundance of HgcAB in the cell lysates is extremely low, Hg(II) was quantitatively

converted to MeHg at sub-nanomolar substrate concentrations. Interestingly, increasing thiol/Hg(II) ratios did not impact Hg methylation rates, which suggests that HgcAB-mediated Hg methylation effectively competes with cellular thiols for Hg(II) consistent with the low apparent KM. Supplementation of CH3-H4folate or pyruvate did not enhance MeHg production, while both ATP and a non-hydrolyzable ATP analog decreased Hg methylation rates in cell lysates under the experimental conditions. These studies provide first insights into the biomolecular processes associated with Hg methylation in anaerobic bacteria.

7. Aminesh Dhara, Ph.D., College of Medicine, University of Kentucky

TgOTUD5, a deubiquitinase regulated by phosphorylation, controls cell cycle progression and maternal turnover in Toxoplasma gondii.

The functions of Ovarian TUmor (OTU) domain containing deubiquitinases (DUBs) are beginning to be explored in Apicomplexa. In this study we functionally characterize a cell cycle regulated DUB, TgOTUD5. HA-tagging of TgOTUD5 revealed a cytoplasmic localization with cell cycle dependent protein expression which is the lowest at M/C boundary, increasing with the progression of cytokinesis and peaking in G1. Repeated failed attempts in isolating a Knockout line suggest its essentiality. Conditional knockdown under the TET-regulated promoter reduces the levels below the detection limit within 24 h resulting in a delay in cell cycle progression accompanied by defective breakdown of maternal remnants following 2 days of ATc treatment. While an E. coli expressed recombinant TgOTUD5 enzyme showed poor DUB activity, insect cell expressed enzyme exhibited markedly enhanced activity suggesting a potential role for post-translational modification. Treatment with lambda phosphatase reduced the efficiency of the DUB activity suggesting phosphorylation plays an important role in regulating TgOTUD5 enzyme activity which could make it a potential drug target.

8. Lu Han, Ph.D., Oak Ridge National Laboratory

Mechanically robust 3D-printed silica part: Binder development for binder jet 3D printing

Binder jet 3D printing is a process in which powder material is deposited layer-by-layer and selectively joined in each layer with ink-jetted binder. This additive manufacturing (AM) process is capable of shaping a variety of materials such as metals, sands and ceramics. Binder jet AM provides significant freedom of geometric design in comparison with traditional manufacturing methods. When compared to other 3D printing methods, binder jet 3D printing does not employ melting or welding during the build process, which saves time and energy, uses less material, provides isotropic properties, and allows for printing of larger parts with lower costs. The bonding strength between the binder and the printed material is a critical issue for binder jet printing and limits the geometries that can be printed. In this work, a novel binder for silica was developed and printing parameters such as different saturation and binder content were carefully investigated. The novel binder provides an exceptionally high strength in sand parts in particular, which were found to be stronger than unreinforced concrete. Furthermore, the binder can be washed, which is ideal for sand-casting molds and washout tooling. Thus, to better understand the mechanism of adhesion for the strong binder, the combination of time-dependent NMR, FT-IR, DSC and XPS studies were conducted and they revealed the structure change during curing. Interface structure between silica and polymer binder was studied by Sum frequency generation spectroscopy, which showed a strong interaction between polymer binder and silica after curing. This study to

understanding the mechanisms driving the performance of this binder will aid in developing other binders for the binder jet process.

9. Kendra Hargis-Staggs, Ph.D., College of Medicine, University of Kentucky

Ceruplasmin in aging and Alzheimer's disease neurovascular units

Aging and being female are key unmodifiable risk factors for sporadic Alzheimer's disease (sAD), yet the mechanisms through which either risk factor communicates its increased risk are not clear. Searching across sAD brain transcriptional profiles, we identified a set of robust transcriptional changes that were not only consistent across different labs and measurement platforms, but also showed exacerbated signal in female vs male sAD patients. Ceruloplasmin was found to be among the strongest of these gene expression changes. CP has two splice variants, secretory (sCP) and membrane-bound (mCP). Although sCP is well-characterized, mCP is not, although it is strongly expressed in neurovascular units (NVUs). Prior data suggested that mCP may be more strongly expressed in white matter than gray matter. In preliminary data, we used laser capture microdissection to show that sCP is indeed robustly enriched in white matter. Presently, we are assessing RNA quality in post mortem brain specimens to identify the strongest candidates for laser capture microdissection and analysis of CP expression in gray and white matter NVUs.

10. Nathaniel Holcomb, College of Medicine, University of Kentucky

*ATR I774Yfs*5 generates micronuclei and extra-cellular DNA without impacting viability: a potential driver of carcinogenesis*

Colorectal carcinoma (CRC), the second leading cause of cancer death in the US, claimed the lives of over 50,000 Americans in 2017. We have identified a mutation in the ATR gene relevant to CRC that promotes chromosomal damage and micronuclei formation. This mutation is overrepresented in CRC, yet how it contributes to CRC development is unknown. Transfection of the mutation, ATR-I774Yfs*5, into CRC cells produced abundant micronuclei without impacting cell growth or viability. Multiple CRC lines harboring this mutation endogenously have been identified. These cell lines exhibit the micronucleus phenotype, confirming that the phenotype is not an artifact of overexpression via transfection. This mutant ATR appears to impact replication fork progression, and replicative fidelity is required for micronuclei development, implicating replication in the observed genomic instability produced by ATR-I774Yfs*5. Additionally, cells transfected with ATR-I774Yfs*5 have a higher mutagenic rate than wildtype ATR-transfected cells. All of these findings point to ATR-I774Yfs*5 as a potentially new oncogenic mutation in the development of colorectal cancer.

11. Sam Hughes, College of Medicine, University of Kentucky

Sustained Bupivacaine Analgesia Following Facial Reconstructive Surgery: A Study of Release Kinetics and Injectability of an In situ *Forming Implant (ISI)*

Introduction: Post-operative analgesia for facial reconstructive surgery often relies on opioids. The heightened risk for dependence and other adverse outcomes suggests a need for an alternative method of analgesia. The purpose of this study is to formulate a sustained-release *In situ* forming implant (ISI) that will provide a prolonged nerve block with the use of the local anesthetic bupivacaine. Methods: A drug delivery system was synthesized with drug-loaded poly(β-amino ester) (PBAE) microparticles inside in situ forming poly (lactic-co-glycolic acid) (PLGA). Release kinetics of different combinations of PBAE macromer were compared to calculate bupivacaine

concentration at several time points. A compression apparatus was used to calculate time needed to inject 0.5mL of the different ISIs at various applications of force with different needle gauges available in the operating room. Results: The ISI made with AH6 3:1 PBAE microparticles demonstrated release kinetics most closely approaching the goal, maintaining a steady release of ~115 hours (goal 168 hours), though release plateau was less than the therapeutic dose (<0.15mg/ml). This ISI also demonstrated the most promising injection profile, overall requiring the least time to inject 0.5mL at 25N with a 20G needle. Conclusion: An ISI made with AH6 3:1 PBAE microparticles demonstrated improved release kinetics compared to those previously described in the literature and had a promising injection profile. Further studies are warranted to demonstrate sustained release of bupivacaine at therapeutic concentration and with smaller needle gauges.

12. Magda Javakhishvili, College of Agriculture, Food, and Environment, University of Kentucky

Parental Vigilance, Low Self-control, and Internet Dependency Among Rural Adolescents

With increased access to the internet worldwide, there have been unprecedented new opportunities, but also challenges, associated with internet-related experiences. In particular, adolescents, who are known for their heightened risk for delinquent and health-compromising behaviors (e.g., Rensick et al., 1997), along with developing autonomy from parents, yet with still rather poor decision making, may be equally or more vulnerable to potentially harmful online influences and to experiencing internet-related dependency. Dominant theoretical frameworks attempting to explain internet dependency emphasize underlying psychopathological mechanism (e.g., Bell et al., 2005; Chou & Hsiao, 2000; Davis, 2001; Ha et al., 2007; Kraut et al., 1998; Ko et al., 2009; Shapira et al., 2003;). However, the current study uses a more behavioral approach, identifying individual differences in low self-control (Gottfredson & Hirschi, 1990) as explaining variability in dependency. Informed by Gottfredson and Hirschi's (1990) theoretical insights as well as empirical evidence from the developmental literature (e.g., Zuckerman & McDaniel, 2003), the current study tests direct and indirect effects by low self-control on adolescent internet dependency. Specifically, it examines whether parenting and parental internet monitoring have significant direct effects on adolescent internet dependency and whether low self-control mediates this link, net any effects by age, sex, or socio-economic status. The sample included N = 620 9th to 12th graders (age range = 14 to 19 years, M = 15.91) in a rural county in the Southeastern United States who completed a survey study on emotional and behavioral risks associated with problematic and aggressive online behaviors. Approximately 46% were males, and 54% females; 51% lived with two biological parents, whereas 49% in other living situations. In addition, 80% were European American, 8.3% African American, 7.5% Hispanic American, and the remaining 4.2% included Asian American, American Indian, Pacific Islander, or other. Internet dependency was assessed by the Internet-Related Experiences Questionnaire (Beranuy et al., 2009), which measures how emotionally and behaviorally dependent a person is onto internet use, for instance, whether they give up doing certain things for the sake of internet, whether they feel nervous or worried without, includes 10 items. Low self-control was measured by three well-known scale scores: Low Self-Control short form (Grasmick, Tittle, Bursik, & Arneklev, 1993), Sensation Seeking and Impulsive Control (Zuckerman, Eysenck, and Eysenck, 1978), and the Brief Self-Control Scale (Tangney, Baumeister, & Boone, 2004). Parenting was measured by maternal monitoring and closeness, part of the Adolescent Family Process scale (Vazsonyi et al., 2003), while parental internet monitoring was assessed by three items which indicated whether parents sat with youth while on the internet, or inquired about what they are doing or which websites they

were visiting. All measures demonstrated high reliability. Model specification included paths from parenting constructs to low self-control and from both parenting and low self-control to internet dependency, where fully latent constructs were used. Analyses were completed in Mplus 7.4. (Muthén & Muthén, 2015). Significance of indirect effects was evaluated using 95% bias-corrected bootstrapped confidence intervals, and exact p-values. Findings (shown in Figure 1) revealed that low self-control was the strongest and only significant predictor of adolescent internet dependency, uniquely explaining 21.4% of variance. No significant direct effects by parenting variables on adolescent internet dependency were found; however, consistent with study hypotheses, low self-control mediated the link between parental internet monitoring and internet dependency, net any effects by parenting (coefficient = -.29, p = .024, with a 95% BCI [-.616; -.09]. The same did not hold for parenting (closeness and general monitoring). Study findings highlight both the salience of low self-control in understanding adolescent internet dependency as well as positive indirect effects from parental internet monitoring in particular.

13. **Selvakumar Jayaraman, Ph.D.**, College of Science and Mathematics, Wright State University

Redox Non-Innocent dithiolene-N-Heterocyclic carbene complexes and its catalytic application

Transition metal dithiolene complexes have been notably successful complex for the past five decades for a variety of potential applications including sensing, photophysical, superconducting, hydrogen evolution catalysts and recovery of alkenes from petroleum feedstocks. Intriguing structural characteristics coupled with interesting electrochemical properties makes this class of complexes attractive for modern researchers. N-heterocyclic carbenes (NHC) are one of the most interesting ligands with limitless applications in organic- and organometallic chemistry. We are interested in exploring new possible catalytic use of dithiolene-NHC hybrid complexes derived from combine these two versatile complexes. In this contest, we have developed the first electrochemical release of NHC from redox non-innocent dithiolene-N-heterocyclic carbene complex and subsequent organocatalysis reaction of cinnamaldehyde to butyrolactone also we have established the first dithiolene catalyzed 1,3-dipolar cycloaddition reaction of ethyl \

14. **Eleanor Johnson**, College of Medicine, University of Kentucky

Progesterone Pretreatment Decrease Acute Stress Effect on Cognition and Sgk1 Expression in Sprague-Dawley Rats

Stress is highly prevalent, has negative health consequences, and causes deficits in cognitive function. The impact on cognition is thought to be exerted, at least in part, through stress-induced glucocorticoid action in the brain. Serum and glucocorticoid kinase 1 (Sgk1) is a key downstream effector of glucocorticoid's actions. Progesterone antagonizes glucocorticoid signaling at transcriptional and allosteric modulating levels. Here, we investigated if progesterone pretreatment alleviates the cognitive deficits and elevated Sgk1 associated with acute restraint. Thirty-one adult Sprague-Dawley rats (21 males/ 10 females) were trained in the Morris Water Maze. The rats were placed into one of four groups: 1) stressed and progesterone (n=8), 2) unstressed and progesterone (n=7), 3) stressed and vehicle (n=7), and 4) unstressed and vehicle (n=9). After each of the three training days, progesterone-treated groups were dosed with progesterone (10 mg/ kg). On day 4, a 3 hour restraint was applied immediately prior to the water maze probe trial. Progesterone had no effect on training in any group, and had no effect on probe trial performance in unrestrained animals. In restrained animals, stress caused a significant decrease in vehicles that was reversed by progesterone. In a subset of animals (n=20; stressed

and progesterone (n=6); unstressed and progesterone (n=4); stressed and vehicle (n=4); and unstressed and vehicle (n=6), Western blot analysis of hippocampal tissue revealed a non-significant trend towards upregulation of SGK1 with stress, and suppression of that Sgk1 response in stressed, progesterone-treated animals. This data suggests progesterone may have some effect on decreasing stress response.

15. Sanjay Joshi, College of Agriculture, Food and Environment, University of Kentucky

The Role of Transcription Factor LBD40 in Arabidopsis *Embryogenesis*

Somatic Embryogenesis (SE) is an artificial process by which an embryo is derived from a single somatic cell or group of somatic cells which is regulated by key transcription factors (TF), including AGAMOUS-like 15 (AGL15). SE is a valuable means to regenerate transgenic plants to meet food demands or test gene function, but is poorly understood. Therefore, understanding regulatory mechanisms in SE is fundamentally important. One of the intriguing proteins with which AGL15 interacts is LBD40. LBD40 encodes a LATERAL ORGAN BOUNDARIES (LOB)-domain TF that is unique to plants and is specifically expressed during seed development, primarily in the embryo. The main objective of the research is to understand the mechanism of embryogenesis in Arabidopsis, specifically focusing on interaction between AGL15 and LBD40 in planta. Siliques and embryo culture tissue with epitope tagged transgenes are used for Chromatin-Immuno Precipitation (ChIP). The DNA binding regions of these proteins are assessed using ChIP-sequencing. In-vivo protein interaction of AGL15-LBD40 has been found using Co-immunoprecipitation. RNA seq results of 7-8 days old seeds from a lbd40/41 mutant line showed 335 genes as significantly expressed targets. The Gene Ontology (GO) enrichment analysis showed overrepresentation of biological processes that are associated with SE, suggesting importance of LBD40 in SE. The ChIP-Seq experiment along with RNA-seq of LBD40 will help us to understand better somatic embryogenesis.

16. Samudu Karunadasa, College of Agriculture, Food and Environment, University of Kentucky

Cytokinin signaling promotes misfolded protein accumulation by modulating the protein synthesis rates in Arabidopsis thaliana.

Cytokinins are important in plant growth and development and are known to modulate environmental stress responses through a complex hormonal interplay. This study aims at uncovering the relation of cytokinin and misfolded protein accumulation through determining 1) the sensitivity of cytokinin-related plant lines to different antibiotics which affect protein translation 2) the misfolded protein levels in cytokinin-related lines in the presence of antibiotics that cause protein mistranslation 3) the protein synthesis rates in cytokinin-related plant lines. Transgenic plant lines with increased activity of the cytokinin response activator ARR1, were assessed in the presence of different aminoglycoside antibiotics, which are known to affect protein translation. ARR1 gain-of-function transgenics showed a higher sensitivity to antibiotics that promotes protein mistranslation (eg: kanamycin), but they were tolerant to aminoglycosides that cause complete protein translation inhibition (eg: spectinomycin). This suggests that increased cytokinin action promotes protein synthesis rates which in turn promotes misfolded protein accumulation.

17. Christine Kim, School of Medicine, University of Louisville

Assessing the role of arsenite in disrupting the EGFR signaling axis

The epidermal growth factor receptor (EGFR) is a receptor tyrosine kinase localized on the cell surface. Overexpression of EGFR has been used as biomarkers for many different types of cancers, including lung cancer. Though there is a strong association between arsenic and lung cancer development, the clear mechanism is unknown. We hypothesize that chronic arsenite exposure disrupts the EGFR endocytic trafficking, leading to increased receptor expression. The goal of this project is to study impact of chronic exposure to "a physiologically relevant" level of arsenite on the EGFR expression, distribution and trafficking. A non-malignant human bronchial epithelial cell line, Beas-2B cells were exposed to 100nM sodium arsenite for 24 weeks. The chronic arsenite-treated cells had increased EGFR protein expression levels and activity, increased transcription levels of TGFα, and altered the distribution of the EGFR. In conclusion, the impact of chronic arsenite exposure on the EGFR signaling axis can explain arsenite-induced overexpression of the EGFR that is commonly characterized in lung cancer.

18. Geoffrey Li, Ph.D., School of Medicine, Vanderbilt University

Towards the Structural Characterization of Peripheral Myelin Protein 22 and Its Mutants in Model Membranes by NMR Spectroscopy

Peripheral myelin protein 22 (PMP22) is a 160-residue tetraspan integral membrane protein that is highly expressed in myelinating Schwann cells. It is known to regulate Schwann cell proliferation and myelin production. Genetic alterations, such as point mutations or gene duplication, of PMP22 cause severe dysmyelination of peripheral nerves, a condition known as Charcot-Marie-Tooth Disease (CMTD), for which no treatment is yet available. To date, there is no experimentally determined structure of PMP22 except for a homology-based model based on the crystal structure of claudin-15. Our long-term goal is to determine the structure of PMP22 in model membrane using NMR. We acquired NMR spectra for PMP22 in different micelles and bicelles to screen for optimal conditions for NMR. We found that the NMR spectra of PMP22 solubilized in neutral alkylglycoside detergents gave the most resolved and dispersed peaks. We also compared the NMR spectra of two CMTD mutant forms of PMP22: L16P (Trembler-J phenotype) and G150D (Trembler phenotype) with that of the wild type protein. We found a significant difference in the spectral pattern of the mutants compared to the wild-type protein suggesting a change in structure and dynamics. Our findings will illuminate structural bases both for the function of properly folded PMP22 and for the misfolding of the mutant forms of PMP22 that promote CMTD

19. Zulong Liu, Ph.D., College of Medicine, University of Kentucky

Targeting ABL Kinases Reverses Resistance to BRAF Inhibitors: A Potential New Therapy For Treatment-Refractory Melanomas

Melanoma is a highly aggressive and deadly cancer once it has metastasized, and although new therapies increase life-span, the 5-year survival rate is still low (15-20%). Treatment of patients with immunotherapy is curative for a subset of patients; however, a significant fraction (30-40%) do not respond. Fifty-sixty percent of melanomas harbor BRAF mutations (e.g. V600E), which drive disease development and progression. Selective inhibition of the mutant BRAF protein with BRAF inhibitors (BRAFi) such as vemurafenib (PLX) is a highly promising therapeutic approach. However, despite remarkable clinical responses, nearly all patients develop resistance to the drug and succumb to their disease. To identify a new target and to develop new therapeutic

approaches to reverse resistance, we examined whether ABL family non-receptor tyrosine kinases (ABL1, ABL2; a.k.a. Abl, Arg) are activated following resistance and play a role in resistance, since previous data from our laboratory demonstrated that BRAF cooperates with Src Family Kinases (SFKs) to activate ABL1 and ABL2, which then subsequently promote metastatic progression. Importantly, ABL1 mRNA is significantly increased in melanomas from patients who progress following BRAF inhibitor (BRAFi) treatment. Moreover, ABL1 and ABL2 kinase activities are potentiated by Src Family Kinases in cell lines that have developed resistance to BRAFi. Furthermore, ABL1 expression is increased in the cytoplasm and decreased in the nucleus in resistant cells, consistent with previous data indicating a transforming role for cytoplasmic ABL1 (as opposed to a pro-apoptotic role for nuclear ABL1). Significantly, ABL1/ABL2 activation during resistance has biological consequences as inhibition of ABL1/2 using nilotinib (FDA-approved 2nd generation TKI), ponatinib (3rd generation TKI) or GNF-5 (highly specific allosteric inhibitor) dramatically reverses resistance to BRAFi in colony formation and cell viability assays and induces apoptosis. To identify the mechanism by which the drugs reverse resistance, we examined activation of ERK-dependent signaling pathways as well as markers for the EMT (epithelial-mesenchymal-transition)-transcription factor (TF) switch (ZEB2/SLUG->ZEB1/TWIST1), which promotes invasion and resistance in melanoma cells. Importantly, resistant cells had reactivation of a MEK/ERK/FRA/MYC signaling pathway, as well as induction of the EMT-TF switch and N-cadherin expression, and treatment with nilotinib or GNF-5 potently blocked activation of these pathways. Significantly, targeting ABL1/ABL2 with nilotinib also dramatically reversed BRAFi-resistance, in vivo. Taken together, our data suggest that targeting Abl kinases may be a very promising strategy to reverse BRAFi resistance in melanomas harboring mutant BRAF. Moreover, our data also are likely to have relevance to other aggressive cancers that harbor mutant BRAF (e.g. colon, lung).

20. Siavesh Mazdeyasna, College of Engineering, University of Kentucky

Noninvasive Intraoperative 3D Imaging of Blood Flow Distributions in Mastectomy Skin Flaps

A novel speckle contrast diffuse correlation tomography (scDCT) was recently developed in our laboratory as a noninvasive and noncontact optical imager for 3D imaging of blood flow (BF) distributions in relatively deep tissues (up to 10 mm). The scDCT uses a galvo mirror to remotely deliver focused near-infrared point light to source positions and employs a sensitive sCMOS camera to rapidly quantify spatial diffuse speckle fluctuations resulting from moving red blood cells (i.e., BF). This system also integrates an innovative photometric stereo technique with the same camera to obtain tissue surface geometry. The scDCT has been tested in tissue-simulating phantoms, rodent brains, human burns, and mastectomy skin flaps. In a preliminary study using scDCT and a commercial dye-based fluorescence angiography, we observed similar BF patterns on mastectomy skin flap surfaces. The scDCT may provide objective information for the assessment of skin flap viability to prevent skin flap necrosis.

21. Rebecca McGrail, College of Agriculture, Food, and Environment, University of Kentucky

Addressing Root Turnover at Differing Phosphorus Fertility with the Agroecosystem Mesocosm Facility

Mesocosms are experimental systems which simulate naturally occurring processes with greater variable control than field experiments. The Agroecosystem Mesocosm Facility is being utilized to ascertain the soil biogeochemical processes affecting the transformation and fate of organic phosphorous immobilized in legacy roots. Twenty-four mesocosms were constructed with an A

and B horizon from a low P or high P soil. Four treatments were established per P regime: fallow, corn, winter wheat, and corn-wheat rotation. A greater number of roots were placed in the A horizon in low P conditions compared to high P conditions. These roots may serve as a nutrient source for the following crop through decomposition. Images collected in rotational treatments suggest greater decomposition of old roots in the presence of new roots. Overall, the research will provide information necessary to minimize inorganic P inputs by improving our understanding of organic P utilization found in legacy root biomass.

22. Kanthi Nuti, College of Arts and Sciences, University of Kentucky

Charge effects and their role on particle transport in polymeric gels

Biological hydrogels are known to fulfill a number of important physiological functions, serving as lubricants in joints, acting as barriers against pathogens and serving as selective filters for nutrients, proteins, ions and drugs. All biogels are heterogeneous with varied biophysical properties arrayed on spatially disordered polymer networks. Nanoparticles diffusing in such biogels experience a mixture of complex attractive and repulsive interactions. Using fluorescence correlation spectroscopy (FCS), we have systematically examined the role of probe charge and network charge density on transport and dynamics of probe molecules in polymeric gels. Through a combined theoretical and experimental approach, we have previously shown that particle transport in homogeneously charged dextran gels is highly asymmetric. These dextran gels are low density. In this work, we show increasing the net charge on the probe molecule has large consequences on the transport behavior in attractive gels but minimal effects on transport in neutral or repulsive gels. However, asymmetric transport in the high charge density PVA networks is considerably reduced. Increasing the net probe charge, however, results in nearly identical transport behavior in both low and high charge density polymer networks. Initial results on probe transport in polyampholytic PVA and the dependence of hydrophobic interactions on biotinylated probes will also be presented. Knowledge of polymer-prove interactions could serve as a guideline in the rational design of promising therapeutics that must overcome the significant work required to navigate biological hydrogels in vivo to successfully deliver their payloads.

23. Priyanka Paul, Ph.D., College of Agriculture, Food, and Environment, University of Kentucky

The regulatory role of AGL18 in Arabidopsis *embryogenesis*

The molecular mechanisms of Arabidopsis embryogenesis are still largely unknown. A MADS domain transcription factor (TF), AGAMOUS-LIKE15 (AGL15) accumulates mainly, although not solely, during embryogenesis. Ectopic expression of AGL15 stimulates development of somatic embryos from zygotic embryo explants. Previous reports stated that the product encoded by AGAMOUS-LIKE18 (AGL18) was structurally related and showed overlapping expression patterns with AGL15 in Arabidopsis. Overexpression of both AGL15 and AGL18 in Arabidopsis produced analogous phenotypes, such as morphological variations and late flowering time. Here, we showed that like AGL15, constitutively expressed AGL18 promotes somatic embryogenesis from the shoot apical region of seedlings in liquid media containing 2,4-D. Moreover, AGL18 interacts with AGL15 to form a heterodimer by co-immunoprecipitation (Co-IP). In order to characterize the roles of the AGL18 transcription factor complexes at the molecular level, we studied genome-wide the direct targets of AGL18. We used chromatin immunoprecipitation (ChIP) followed by high-throughput sequencing to obtain genome-wide DNA-binding sites of AGL18. The results demonstrate that AGL18 binds to thousands of sites in the genome in two biological replicates. Then we compared ChIP-seq data for AGL15 to the AGL18 data. Both data sets were

generated from embryo culture tissues. A significant number of genes were bound by both AGL15 and AGL18. GO analysis revealed these genes were enriched for seed, embryo and reproductive development as well as hormone and stress responses. The results also suggest that AGL18 regulates its own expression through a positive auto-regulatory loop. The binding of AGL18 to cis-regulatory elements of other MADS-box genes, including AGL15 and AGL16, and expression analyses reveal that this protein is a key component in the regulatory transcriptional network to control embryogenesis in Arabidopsis.

24. Warlen Pereira Piedade, College of Arts and Sciences, University of Kentucky

Siah E3 ubiquitin ligase control of CDHR1a protein stability is required for photoreceptor cell development during zebrafish eye development.

E3 ubiqiutin ligases mediate orderly and precise targeting of protein degradation to maintain biological homeostasis and coordinate proper development. We recently discovered that Siah family of E3 ligases plays a role in early ocular morphogenesis, in particular fusion of the optic fissure. Interestingly, Siah ligases are also expressed during photoreceptor development and are predicted to target CDHR1a, a cadherin superfamily of calcium-dependent cell adhesion molecules and photoreceptor-specific cadherin. Mutations in this cadherin are associated with inherited retinal dystrophies, such as cone-rod dystrophy. Using whole mount in situ hybridization we detected Siah gene expression in the outer nuclear layer and in the retinal ganglion cell layer. CDHR1a expression overlaps with both Siah genes in the outer segment of the retina at 72 and 96hpf using Fluorescence whole-mount in situ hybridization. We therefore hypothesized that Siah regulates CDHR1a during photoreceptor development. To test our hypothesis, we confirmed siah-mediated targeting of CDHR1a for degradation using mammalian cell culture and western blotting analysis. Second, we created two transgenic zebrafish lines that express Siah1 or a dominant negative (Siah□RING) under the control of the heat shock promoter. Using heat shock to induce Siah1 expression at 48 and 60hpf, resulted in a decrease of developing rods and cones at 72 and 96 hpf. Retinal ganglion, amacrine and horizontal cells, were not affected. In addition, siah1 induction resulted in a significant reduction of proliferating cells in the retina. Taken together, our results suggest that Siah ubiquitin ligases may control CDHR1a stability and therefore regulate the photoreceptor cell proliferation and differentiation.

25. Vira Pravosud, College of Public Health, University of Kentucky

Exceptional Survival among Kentucky Stage IV Lung Cancer Patients: Appalachian versus Non-Appalachian Populations

Objective: To determine differences in exceptional survival (ES) between lung cancer patients residing in the Appalachian versus non-Appalachian regions of Kentucky. Exceptional survival was defined as having lived at least five years past diagnosis. Methods: This was a population-based case-cohort study of Kentucky patients, aged 20 years and older, diagnosed with stage IV lung cancer (non-small cell carcinomas) between January 1, 2000 and December 31, 2011. The data were drawn from the Kentucky Cancer Registry. Results: No significant differences were found between the proportions of patients who survived five years and more among residents of the two regions (p=0.2807; 107 [2.5%] versus 254 [2.9%]). Moreover, findings from the multivariable logistic regression model revealed no significant differences between Appalachian and non-Appalachian patients concerning the odds of ES (Adjusted Odds Ratio, AOR: 0.94, 95% CI: 0.72-1.23). Being a woman and undergoing a surgery as the patient's only treatment were associated with higher odds of ES (AOR: 1.81 [1.43-2.29]; AOR: 5.03 [3.37-7.49]), respectively).

Increasing age, having an unspecified histologic type of cell carcinoma, third/fourth or unknown grade of tumor, and undergoing radiation therapy, or receiving a combination of the following treatments: surgery and/or chemotherapy, and/or radiation therapy were associated with decreased odds of ES (AOR: 0.98 [0.97-0.99]; AOR: 0.64 [0.47-0.88]; AOR: 0.67 [0.47-0.96]; AOR: 0.59 [0.42-0.83]; 0.33 [0.23-0.48]; AOR: 0.69 [0.52-0.91], respectively).Conclusion: The Appalachian and non-Appalachian stage IV lung cancer patients did not differ significantly regarding the odds of surviving five years past diagnosis. However, a number of other factors were associated with increased or decreased odds of exceptional survival.

26. Jacqueline Rivas, Ph.D., College of Medicine, University of Kentucky

Reversing interleukin-10 mediated immune suppression enhances anti-tumor immunity in chronic lymphocytic leukemia

B cell Chronic Lymphocytic Leukemia (CLL) is characterized by an accumulation of abnormal B cells, leading to serious immune dysfunction. This immune suppression is partially due to the production of mediators that downregulate T cell responses, and as a result many T-cell-based immunotherapies have experienced limited success in trials with CLL. CLL cells secrete the immunoregulatory cytokine Interleukin-10 (IL-10), and we previously found that eliminating IL-10 signaling in T cells reduced the growth of CLL. Therefore, we investigated the therapeutic potential for IL-10 blockade to enhance anti-tumor CD8+ T cells and increase the efficacy of immunotherapy in CLL. IL-10 production by human and Eμ-TCL1 mouse CLL cells depends on the transcription factor Sp1, and the Sp1 inhibitor mithramycin (MTM) suppresses CLL IL-10 production. However, MTM is not well tolerated in vivo, so we synthesized a novel analogue of MTM (MTM23), which similarly suppresses IL-10 and is tolerated at 12-fold higher doses. MTM23 enhances anti-CLL immunity in vivo by suppressing CLL growth and IL-10 production, allowing for increased CD8+ T cell proliferation and interferon-γ production. Furthermore, adding MTM23 to anti-PD-L1 immunotherapy greatly improved the control of CLL in vivo. CD8+ T cells were more prevalent in double treated mice than anti-PD-L1 alone, with an increase in CD8+ T cell functionality. This paradigm shifting approach is novel as current therapies for CLL do not target IL-10 and it may increase the efficacy of immunotherapies in human CLL. Moreover, this could be applicable to other cancers where T cell suppression plays a role.

27. Ana Ferragut Cardoso, Ph.D., School of Medicine, University of Louisville

Overexpression of hsa-miR-186 induces chromosomal instability in arsenic-exposed human keratinocytes

Arsenic (As) exposure affects >200 million people. Skin is its major target. hsa-miR-186 is overexpressed in squamous cell carcinoma relative to hyperkeratosis. As exposure induces miRNA dysregulation that affects the cell cycle, leading to chromosomal instability. HaCaT transfected with pEP-hsa-miR-186 expression vector (hsa-miR-186 V) or empty vector were maintained with 0 (As-) and 100 nM (As+) As for 2 months. hsa-miR-186 expression levels were assessed by RT-qPCR. Western blot was performed to evaluate BUB1 and CDC27. Numerical and structural chromosomal aberrations were analyzed. hsa-miR-186 expression was elevated in hsa-miR-186 V and slightly higher in hsa-miR-186 V As+. Cells with increased chromosomal number and abnormalities were abundant in hsa-miR-186 V, and more abundant in hsa-miR-186 V As+. Overexpression of hsa-miR-186 and/or arsenic exposure suppressed the levels of BUB1. Deregulation of cell-cycle events leading to aneuploidy may be involved in arsenic-induced carcinogenesis.

28. Melonie Thomas, College of Arts and Sciences, University of Kentucky

Determining the Structure and Stability of Thermoelectric La3-xTe4-Ni Composites using High-Resolution and In-Situ TEM

Lanthanum telluride (La3-xTe4), is a TE material with a figure of merit (zT) of 1.1 at 1275 K. It has been shown that the zT can be increased by 30% when nickel (Ni) nanoparticle (NP) inclusions are introduced to the LaTe1.46. Our goal is to characterize the structures of these high-efficiency TE composites with atomic resolution at ambient and elevated temperatures in high vacuum conditions. When it comes to the stability and performance of these TE composites the La3-xTe4/Ni interfaces play a key factor, but their role and nature are not well understood. The characterization of these TE materials is extremely challenging due to high oxidation of La3-xTe4 which can interfere with the characterization of the interface with Ni. In this work, we devised a method to minimize oxidation during sample preparation and sample transfer between the Focused Ion Beam Scanning Electron Microscope and the Transmission Electron Microscope (TEM). Using this technique, we successfully characterized the interfaces in the TE composite by high-resolution TEM. We further demonstrated that the Ni NPs are directly bonded to LaTe1.46 matrix with the absence of any interfacial layer.

29. Ahamad Ullah, College of Arts and Sciences, University of Kentucky

Systematic characterization of W-doped vanadium dioxide, V1-xWxO2 (x=0.0, and 0.01) for in-situ biasing TEM experiments

Vanadium dioxide (VO2) is a fabulous co-related material which exhibits metal to insulator transition (MIT) coupled with the structural change from the rutile phase (R) to monoclinic phase (M1) at the MIT temperature (T_c^0= 341 K). During the phase transition, the electrical resistivity and optical transparency of VO2 change several orders of magnitude. This unique property makes it a promising material for various applications such as thermo/electrochromics, smart windows, Mott transistors, thermal actuators, and sensors. The T_c^0 in VO2 can be tuned by the introduction of chemical dopants for different application purposes. Yet, the underlying mechanisms of MIT temperature variation induced by the dopants are poorly understood. Using in-situ biasing in a transmission electron microscopy (TEM), we plan to investigate the effect of tungsten (W) doping on the phase transitions of V1-xWxO2 (x=0.0 and 0.01) nanowires/beams, as a function of electrical response. In this study, V1-xWxO2 (x=0.0 and 0.01) nanowires/beams were synthesized hydrothermally at 250 °C for seven days and systematically characterized using powder X-ray diffraction (PXRD), scanning electron microscopy (SEM), electron dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS) and TEM. In the future, V1-xWxO2 (x=0.0 and 0.01) nanowires/beams will be used to carry out in-situ biasing TEM experiments to observe structural transformation of the materials in real time. High-resolution TEM micrographs of the nanowires/beams from in-situ experiments will be further characterized to determine the phase transition in detail.

30. Kristyn VanDerMeulen, College of Arts and Sciences, University of Kentucky

Deciphering the Complex Relationships of Periocular Mesenchyme Subpopulations within the Developing Zebrafish Ocular Anterior Segment

The anterior segment (AS) is a complex collection of structures used to project light onto the retina and maintain inner eye homeostasis. Anterior Segment Dysgenesis (ASD) is a spectrum of developmental disorders effecting these structures and resulting in visual impairment. The neural ectoderm, surface ectoderm, and Periocular Mesenchyme (POM) lineages come together in early development to assemble the AS. We believe that dysregulation of the molecular machinery regulating incorporation of POM into AS tissues may predispose individuals to ASD. Using transgenic zebrafish embryos expressing GFP in POM cells we have discovered the POM comprise of several subpopulations denoted by unique AS distributions, population sizes, and migratory dynamics. Static and live time-lapse analysis clearly defined significant differences amongst various POM cell migrating onto the AS. This suggests that AS formation is multifaceted. In order to better understand AS development, we sought to characterize each subpopulation on a molecular level. To isolate molecular signatures of POM subpopulations (representing cells regulated by FoxC1b, FoxD3, Pitx2 or Sox10), we used FACS and RNA sequencing to compile their unique transcriptomes. A 4-way comparison of the data indicated ~20,000 genes were similarly expressed. However, more than 2,000 genes were uniquely expressed in the POM-specific FoxC1b population, ~4,000 in the neural crest-specific Sox10 population ~5,000 in the Pitx2 subpopulation and ~370 in the FoxD3 population. Detailed analysis of this data is expected to uncover novel marker gene expression patterns for each subpopulation in addition to common regulators of the POM cell lineage.

31. Sathya Velmurugan, Ph.D., College of Medicine, University of Kentucky

Mitochondrial Na+/Ca2+ exchanger inhibition reduces oxidative stress and Ca2+ sparks in diabetic rat cardiac myocytes

Decreased mitochondrial Ca2+ concentration ([Ca2+]m) compromises mitochondrial function leading to cardiovascular complications in type-2 diabetes (T2D). As intracellular Na+ concentration ([Na+]i) is elevated in T2D due to enhanced Na+-glucose co-transport, we hypothesized that the mitochondrial Na+/Ca2+ exchanger (mNCX) is faster in T2D rat hearts, resulting in reduced [Ca2+]m. Rat expressing human amylin in the pancreatic β-cells (HIP rat) was used as a model for late-onset T2D. In HIP rat myocytes vs. wild-type control, (1) mNCX activity was higher at high [Na+]i (³20 mM), (2) Rate of mitochondrial Ca2+ influx was lesser, (3) CGP-37157, a selective mNCX antagonist, and mitoTempo, a mitochondria-targeted antioxidant, lowered the enhanced ROS level, and (4) CGP-37157 reduced the increase in frequency of spontaneous Ca2+ sparks. In conclusion, mNCX activity is enhanced while mCa2+ influx is reduced in HIP myocytes, culminating in reduced [Ca2+]m. Reduction in ROS and Ca2+ spark frequency upon mNCX inhibition suggests that enhanced mNCX activity may contribute to cardiac complications of T2D via oxidative stress and pro-arrhythmogenic Ca2+ sparks.

32. Oliver Voecking, Ph.D., College of Arts and Sciences, University of Kentucky

Single cell analysis of periocular mesenchyme during anterior segment development

Development of the anterior segment (AS) of the eye largely depends on a group of neural crest cells, named periocular mesenchyme (POM). Thus far, only the physiological role of these cells during maldevelopment of AS has been investigated. An understanding about details of their molecular function is largely missing. As such, clinicians lack the opportunity to molecularly screen and treat several diseases associated with the AS, including AS dysgenesis (ASD) and glaucoma. In this study, we used Foxc1b, a known AS determinant, to isolate and characterize POM cell development. A transgenic line of zebrafish, Tg[foxc1b:GFP] was used to isolate AS associated

Foxc1b+ cells and subsequently generate single cell transcriptomes using 10X genomics technology. The great advantage of this technology is that reads from individual cells are tagged with a barcode, allowing to analyze gene expression at a cellular level. By comparing data from different developmental time points, we aim to decipher the POM specification program. Our preliminary data show that there are at least four distinct groups of cells marked by Foxc1b at early stages of AS development. This study provides new insights and novel targets for analysis in how and when the AS forms, while providing new candidate genes for analysis of AS development as well as association with various ASD disorders. This new-found knowledge will be crucial for diagnosis and treatment of ocular diseases such as ASD and glaucoma.

33. Wen Wen, Ph.D., College of Medicine, University of Kentucky

MANF Protects Purkinje Cells from Alcohol-Induced Neurodegeneration

Purpose: Ethanol exposure can lead to significant neurodegeneration in the developing brain due to elevated endoplasmic reticulum (ER)-stress. Mesencephalic astrocyte-derived neurotrophic factor (MANF) is an ER-stress inducible protein expressed in many cell types including neurons. Evidence has shown that MANF can alleviate ER-stress induced cell damage. We hypothesize that MANF may act to maintain ER homeostasis in response to ethanol exposure and deficiency of MANF renders neurons more susceptible to ethanol-induced neurodegeneration. Methods: To test this hypothesis, Purkinje cell specific MANF knockout mice were generated using Cre/Lox recombination. Mice were intubated (gavage) with 5 g/kg/day ethanol or equal volume of H2O once a day for ten days. Locomotor behaviors including open field, rotarods, and balanced beam were tested. The number and morphology of Purkinje cells were examined. Apoptosis was detected by immunohistochemistry of cleaved-caspase 3 and TUNEL assay. ER-stress markers were detected by immunohistochemistry. Results: MANF deficient mice exhibit deficits in the locomotor behaviors after alcohol exposure. Their Purkinje cells showed reduced numbers, shrank cell body, missing dendrite, and increased apoptosis. ER-stress markers including GRP78, ATF6, p-eIF2α and CHOP were upregulated in MANF deficient Purkinje cells after alcohol exposure. Conclusions: MANF deficient Purkinje cells are more susceptible to ethanol-induced neurodegeneration, possibly due to elevated ER-stress, suggesting the neurotrophic role of MANF in protecting neurons from ethanol-induced neurodegeneration is partially through the alleviation of ER-stress.

34. Zhihui Zhu, Ph.D., College of Medicine, University of Kentucky

Ceramide regulates interaction of Hsd17b4 with Pex5 and function of peroxisomes

The sphingolipid ceramide regulates beta oxidation of medium and long chain fatty acids in mitochondria. It is not known whether it also regulates oxidation of very long chain fatty acids (VLCFAs) in peroxisomes. Using affinity chromatography, co-immunoprecipitation experiments, and proximity ligation assays we discovered that ceramide interacts with Hsd17b4, an enzyme critical for peroxisomal VLCFA oxidation and docosahexaenoic acid (DHA) generation. Immunocytochemistry showed that Hsd17b4 is distributed to ceramide-enriched mitochondrial-associated membranes (CEMAMs). Molecular docking and in vitro mutagenesis experiments showed that ceramide binds to the sterol carrier protein 2-like domain in Hsd17b4 adjacent to PTS1, the C-terminal signal for interaction with Pex5, a peroxin mediating transport of Hsd17b4 into peroxisomes. Inhibition of ceramide biosynthesis induced translocation of Hsd17b4 from CEMAMs to peroxisomes, interaction of Hsd17b4 with Pex5, and upregulation of DHA. This data

indicates a novel role of ceramide as molecular switch regulating interaction of Hsd17b4 with Pex5 and peroxisomal function.

35. Zhihui Zhu, Ph.D., College of Medicine, University of Kentucky

Ceramide-induced interaction between tubulin and voltage-dependent anion channel 1 regulates mitochondria ATP release in astrocytes

Ceramide is a cell signaling sphingolipid known to regulate a plethora of biological functions including cell cycle, apoptosis, and mitochondrial function. Recently, mitochondrial ATP release to the cytosol was shown to depend on the interaction of voltage-dependent anion channel 1 (VDAC1) with tubulin. Using crosslinking to the ceramide analog pacFACer and superresolution fluorescence microscopy, we found that ceramide interacts with tubulin and that ceramide-associated tubulin (CAT) is translocated from the perinuclear region to ceramide-enriched and mitochondria-associated membranes (CEMAMs) that are co-distributed with microtubules. This translocation was prevented in astrocytes deficient of neutral sphingomyelinase 2 (nSMase2), an enzyme generating ceramide from sphingomyelin, or cells treated with Fumonisin B1 (FB1), an inhibitor of ceramide synthases. Proximity ligation and co-immunoprecipitation assays showed that ceramide depletion reduced association of tubulin with VDAC1. Ceramide depletion led to higher levels of ATP in astrocytes, suggesting that ceramide-induced CAT formation leads to VDAC1 closure, thereby reducing mitochondrial ATP release. We also show that mitochondrial motility is increased in nSMase2-deficient astrocytes and that amyloid beta peptide (Aβ42) does not induce mitochondrial fragmentation in these cells. These results suggest that CAT regulates mitochondrial motility and that blocking ceramide generation may increase ATP release and protect mitochondria in Alzheimer's disease.

36. Jiaying Weng, College of Arts and Sciences, University of Kentucky

On sparse Fourier transform inverse regression for sufficient variable selection

The big data era poses great challenges as well as opportunities for researchers to develop efficient statistical approaches to analyze massive data. Sufficient dimension reduction (SDR) is such an important tool in modern data analysis and has received extensive attention in both academia and industry. I introduce an inverse regression estimator using Fourier transforms, which is superior to the existing SDR methods in two folds, (1) it avoids the slicing of the response variable, (2) it can be readily extended to solve high dimensional data problem. For the ultra-high dimensional problem, I investigate a minimum discrepancy approach to achieve optimal solutions and also develop a novel and efficient optimization algorithm to obtain the sparse estimates. Simulation studies and real data analysis are carried out to illustrate the superior performance of our proposed methods.

37. Kai Tan, College of Arts and Sciences, University of Kentucky

COM-negative binomial distribution: modeling overdispersion and ultrahigh zero-inated count data

We focus on the COM-type negative binomial distribution with three parameters, which belongs to COM-type (a; b; 0) class distributions and family of equilibrium distributions of arbitrary birth-death process. Besides, we show abundant distributional properties such as overdispersion and under-dispersion, log-concavity, log-convexity (innate divisibility), pseudo compound Poisson, stochastic ordering, and asymptotic approximation. Some characterizations including sum of equi-correlated geometrically distributed random variables, conditional distribution, limit distribution of COM-negative hypergeometric distribution, and Stein's identity are given for theoretical properties. COM-negative binomial distribution was applied to overdispersion and ultrahigh zero-inated data sets. With the aid of ratio regression, we employ maximum likelihood method to estimate the parameters and the goodness-of-t are evaluated by the discrete Kolmogorov-Smirnov test.

38. Melonie Thomas, College of Arts and Sciences, University of Kentucky

Determining the Structure of Heteroatom Doped Carbon Nano-Onions for Catalysis via Aberration-Corrected STEM

Carbon nano-onions (CNO) are carbon nanostructures with a hollow core and concentric graphitic shells, which can be synthesized at a low-cost and is highly efficient in catalytic reactions. With the presence of many active sites due to the large surface area, CNO have shown promising high efficiencies in many catalytic reactions. Doping CNO with heteroatoms is an effectual method of changing its physical and chemical properties. N and B doped CNOs have shown enhanced catalytic activity in styrene epoxidation reactions and oxygen reduction reaction when compared with undoped CNO. Therefore, the configurations and locations of the incorporated dopant atoms must likely be a key factor determining the catalytic activity of heteroatom doped CNO. Here, for the first time, we show the direct imaging of heteroatoms in N and S doped CNO, via aberration-corrected Scanning Transmission Electron Microscopy equipped with Electron Energy Loss Spectrometer. Furthermore, we provide statistical analysis of dopant configuration and location (with respect to discontinuity/defect sites in graphitic shells) of the three samples, S doped CNO, N doped CNO, and S & N co-doped CNO.