Basic Sciences - Faculty

BMS1: Elevated Cortisol Concentrations in Endurance Athletes Promotes Changes in Bone Mineral Density Through Gene Expression Changes in Osteoblasts

BMS2: Voluntary Wheel Running Attenuates Urinary Bladder Hypersensitivity and Dysfunction Following Neonatal Maternal Separation In Female Mice

BMS3: Deficiency in the Heat Stress Response Could Underlie Susceptibility to Metabolic Disease

BMS4: Novel Models to Evaluate the Human Xenobiotic-Response: Virally Transduced Pregnane X Receptor Knockout Primary Hepatocytes and a 'Humanized' Pregnane X Receptor Mouse

BMS5: Upregulation Of RGS2: A New Mechanism For Pirfenidone Amelioration Of Pulmonary Fibrosis

Basic Sciences - Students

BMS6: Measuring the Relationship between S. mutans Levels and Dental Caries Severity in Patients

BMS7: Insights Into Dhp Induced Cortisol Response In Head Kidney Of Rainbow Trout (Oncorhynchus Mykiss)

BMS8: Serum 25-Hydroxyvitamin D Concentration is Inversely Associated with Mucosal Inflammation in Patients with Ulcerative Colitis

BMS9: Human Behaviors Mediate Spread of Lyme Disease Pathogen, Borrelia burgdorferi, by Increasing Rodent Reservoir Host Activities

BMS10: Potential Treatment of Iron Overloading: Reduced Ttc7 Expression Leads to Robust Urinary Iron Excretion

BMS11: A New Mechanism of Action of Dipeptidyl Peptidase-4 (DPP-4) Inhibition on Prevention of Western Diet Induced Aortic Stiffness in female C57-BL6/J mice

BMS12: The Production, Purification, and Post-Translational Modification of PvdJ Module 2 in Pseudomonas Aeruginosa

BMS13: Coordinate Action of Distinct Sequence Elements Localizes Checkpoint Kinase Hsl1 to The Septin Collar At The Bud Neck In Saccharomyces cerevisiae

BMS14: Identification of Human FANCD2 Structural Domain(s) Integral to DNA Repair via Interaction with Bloom Helicase

BMS15: SOX9 Is Targeted for Proteasomal Degradation by The E3 Ligase FBW7 In Response To DNA Damage

BMS16: Interferon-γ Inhibits Vaccinia Virus Replication and Spread In Lung Epithelial Cells By Inhibiting Actin Polymerization

BMS17: Neuroendocrine Disruption by 17α-Ethinylestradiol and Bisphenol-A in Zebrafish

Clinical Sciences - Students

CS1: A Mouse Model to Assess Neointimal Formation Following Endothelial Denudation And Bioresorbable Stent Implantation

CS2: Effect of Probiotics on the Acute Stress Response

Clinical Sciences - Residents

CS3: Self-Administered Pneumatic Insufflation for the Treatment of Subjective Tinnitus.

CS4: Medical Clearance of the Clinically Medically Stable Psychiatric Patients in the Emergency Department

CS5: Predictive Values of Parathyroid Surgery In A Community Hospital In Southern Missouri

CS6: Re-Treatment of Generalized Granuloma Annulare with Adalimumab

Medical Education - Faculty

ME1: Show & Tell - Some of My Online Worksheets and Ideas for The Future

Medical Education - Students

ME2: Comparison of Umbilical Cord Occlusion Methods: Radiofrequency Ablation Versus Laser Photocoagulation

Medical Education - Residents

ME3: A Comparison of Three Methods for Ossicular Chain Reconstruction

ME4: A Case of Multiple Sclerosis Presenting as Wernicke's Encephalopathy

Basic Sciences - Faculty

BMS1: Elevated Cortisol Concentrations in Endurance Athletes Promotes Changes in Bone Mineral Density Through Gene Expression Changes in Osteoblasts

Bradley Creamer, PhD; Alla Barry, MD

Decreases in bone mineral density (BMD) have been positively associated with orthopedic injuries such as fractures and breaks in endurance athletes. Numerous studies have shown that physical stresses, such as exercise, stimulate bone remodeling, and can lead to sustained changes in bone structure and function. Chronic increases in circulating cortisol, a glucocorticoid secreted via the hypothalamic-pituitary-adrenal axis during times of exercise-induced stress, are associated with decreased BMD through changes in osteoblast functions. In this study, we evaluated the role of exercise-induced stress on circulating cortisol levels in athletes participating in endurance or weight-bearing exercises. In addition, we examined changes in osteoblast gene expression in eight genes thought to be associated with bone remodeling following in-vitro exposure to chronic vs acute, as well as varying levels of glucocorticoids. Overall, salivary cortisol levels were found to be significantly higher in endurance trained athletes at all-time points (prior to, during, and following exercise) than in power-trained athletes. Increased expression of pro-resorption genes Alkaline Phosphatase (ALPL), Dickkoph WNT Signaling Pathway Inhibitor 1 (DKK-1), and RANKL following glucocorticoid treatment in osteoblasts, along with decreased expression of pro-remodeling genes Osteocalcin (OCN) and Osteoprotegerin (OPG), indicated that increased levels of circulating cortisol associated with endurance exercise may play a direct role in bone remodeling and overall BMD. Additional studies examining global changes in gene expression will allow for a deeper understanding of the role of stress and stress related hormonal changes on BMD in athletes. This may provide opportunities to decrease fractures in athletes with decreased BMD.

BMS2: Voluntary Wheel Running Attenuates Urinary Bladder Hypersensitivity and Dysfunction Following Neonatal Maternal Separation In Female Mice

Angela Pierce, PhD; Olivia Eller-Smith, BS; Julie Christianson, PhD

Urogenital pain patients commonly report adverse events early in life, which has been shown to disrupt proper functioning of hypothalamic-pituitary-adrenal (HPA) axis, thereby permanently altering stress responses and the perception of pain. Voluntary exercise has been shown to attenuate many of the negative outcomes associated with HPA axis dysfunction; however, the therapeutic potential of exercise for improving urogenital pain and dysfunction or comorbid mood disorders arising from early life stress has not been determined. Therefore, we housed female mice that did (NMS) or did not (naïve) undergo daily neonatal maternal separation from

postnatal day 1-21, in cages with free access to running wheels to determine if exercise could prevent urogenital hypersensitivity and dysfunction. Sedentary (-Sed) NMS mice displayed significantly increased visceromotor response (VMR) during urinary bladder distension (UBD), had an increased bladder sensitivity, void frequency, and mast cell degranulation compared to naïve-Sed controls. Sucrose preference was increased in NMS-Sed mice and corticotropin-releasing factor receptor 1 (CRF1) and glucocorticoid receptor mRNA levels were significantly reduced in the hippocampus. Early exercise normalized bladder sensitivity, micturition output, and increased brain-derived neurotrophic factor (BDNF) mRNA levels in the hippocampus of NMS mice. Mast cell degranulation was also normalized in NMS bladders following exercise. Taken together, these findings provide novel insight on the efficacy of exercise, an easily translatable clinical intervention, as a potential treatment strategy for chronic urogenital hypersensitivity, neuroimmune dysfunction and behavioral outcomes arising from early life stress.

BMS3: Deficiency in the Heat Stress Response Could Underlie Susceptibility to Metabolic Disease

Robert Rogers, PhD; Matthew Morris, PhD; Joshua Wheatley, MS; Ashley Archer, PhD; Lauren Cook, PhD; Steve Britton, PhD; John Thyfault, PhD; Paige Geiger, PhD

Heat treatment (HT) effectively prevents insulin resistance and glucose intolerance in rats fed a high-fat diet (HFD). The positive metabolic actions of heat shock protein 72 (HSP72), which include increased oxidative capacity and enhanced mitochondrial function, underlie the protective effects of HT. The purpose of this study was to test the ability of HSP72 induction to mitigate the effects of consumption of a short-term 3-day HFD in rats selectively bred to be low-capacity runners (LCRs) and high-capacity runners (HCRs)-selective breeding that results in disparate differences in intrinsic aerobic capacity. HCR and LCR rats were fed a chow or HFD for 3 days and received a single in vivo HT (41°C, for 20 min) or sham treatment (ST). Blood, skeletal muscles, liver, and adipose tissues were harvested 24 h after HT/ST. HT decreased blood glucose levels, adipocyte size, and triglyceride accumulation in liver and muscle and restored insulin sensitivity in glycolytic muscles from LCR rats. As expected, HCR rats were protected from the HFD. Importantly, HSP72 induction was decreased in LCR rats after only 3 days of eating the HFD. Deficiency in the highly conserved stress response mediated by HSPs could underlie susceptibility to metabolic disease with low aerobic capacity.

BMS4: Novel Models to Evaluate the Human Xenobiotic-Response: Virally Transduced Pregnane X Receptor Knockout Primary Hepatocytes and a 'Humanized' Pregnane X Receptor Mouse

Jeff Staudinger, PhD

Pregnane X receptor (PXR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors and functions as a master-regulator of drug-inducible gene expression in the liver and intestine. Ligand-inducible PXR-target genes include key members of the cytochromes P450 (CYP) superfamily that are responsible for the oxidative metabolism of many diverse clinically prescribed drugs. This type of CYP induction can lead to clinically relevant and sometimes life-threatening drug-drug interactions. The standard model to evaluate CYP induction is primary cultures of human hepatocytes. However, due to the limited availability and inter-individual variability associated with primary human hepatocytes, it is necessary to develop new models to observe the human xenobiotic response. Primary cultures of PXR Knockout (PXR-KO) mouse hepatocytes transduced with anadenovirus that encodes human PXR (hPXR) provides one model, and a 'humanized' PXR transgenic mouse represents another. The xenobiotic responses were compared in these two models treated with prototypical human and mouse ligands using real-time quantitative PCR (rt-QPCR). Our data suggest that primary cultures of hPXR-virally-transduced KO hepatocytes or 'humanized' mouse hepatocytes may be appropriate models to study the human xenobiotic response for studies of drug metabolism, as well as drug-drug, food-drug, and herb-drug interactions.

BMS5: Upregulation of RGS2: A New Mechanism For Pirfenidone Amelioration Of Pulmonary Fibrosis

Dennis Wolff, PhD; Yan Xie, MD, PhD; Haihong Jiang, MD, PhD; Suneet Mehrota, PhD; Peter Abel, PhD; Myron Toews, PhD; Stephen Rennard, MD; Reynold Panettieri, Jr, MD; Thomas Casale, MD; Yaping Tu, PhD

Background Pirfenidone was recently approved for treatment of idiopathic pulmonary fibrosis. However, the therapeutic dose of pirfenidone is very high, causing side effects that limit its doses and therapeutic effectiveness. Understanding the molecular mechanisms of action of pirfenidone could improve its safety and efficacy. Because activated fibroblasts are critical effector cells associated with the progression of fibrosis, this study investigated the genes that change expression rapidly in response to pirfenidone treatment of pulmonary fibroblasts and explored their contributions to the anti-fibrotic effects of pirfenidone. Methods We used the GeneChip microarray to screen for genes that were rapidly up-regulated upon exposure of human lung fibroblast cells to pirfenidone, with confirmation for specific genes by real-time PCR and western blots. Biochemical and functional analyses were used to establish their anti-fibrotic effects in cellular and animal models of pulmonary fibrosis. Results We identified Regulator of

G-protein Signaling 2 (RGS2) as an early pirfenidone-induced gene. Treatment with pirfenidone significantly increased RGS2 mRNA and protein expression in both a human fetal lung fibroblast cell line and primary pulmonary fibroblasts isolated from patients without or with idiopathic pulmonary fibrosis. Pirfenidone treatment or direct overexpression of recombinant RGS2 in human lung fibroblasts inhibited the profibrotic effects of thrombin, whereas loss of RGS2 exacerbated bleomycin-induced pulmonary fibrosis and mortality in mice. Pirfenidone treatment reduced bleomycin-induced pulmonary fibrosis in wild-type but not RGS2 knockout mice. Conclusions Endogenous RGS2 exhibits anti-fibrotic functions. Upregulated RGS2 contributes significantly to the anti-fibrotic effects of pirfenidone.

Basic Sciences - Students

BMS6: Measuring the Relationship between S. mutans Levels and Dental Caries Severity in Patients

Neha Bang, BS; Gerald Schlink, PhD

Bacteria living in the oral cavity, such as Streptococcus mutans, can ferment sugars to produce acids, which can damage teeth. Dental caries currently affects more than two billion people worldwide. Consensus has not yet been reached regarding how strongly S. mutans contributes to dental caries: some researchers reason that S. mutans is the primary contributor to dental caries, while others claim that other species may also be significant contributors. The purpose of this study was to determine if there was a correlation between the S. mutans UA159 concentration and the plaque index in 24 patients at the Missouri Southern Dental Hygiene Clinic. S. mutans DNA was extracted from each dental plaque sample. A qPCR assay was used to quantify levels of dexA, a gene coding for dextranase, which hydrolyses glucans in plaque. The concentration of dexA correlated with the concentration of S. mutans in each sample. We hypothesized that there would be a strong correlation between the S. mutans level and dental caries severity. Linear regression analysis indicated that there was no correlation between the S. mutans concentration and plaque index (R2 = 0.0248). There was no correlation between the threshold cycle value and plaque index (R2 = 0.0039). Our results suggest that other S. mutans strains or bacterial species contributed more strongly to dental caries than did S. mutans UA159.

BMS7: Insights into Dhp Induced Cortisol Response in Head Kidney Of Rainbow Trout (Oncorhynchus mykiss)

Callie Edwards, MSI; Scott Lankford, PhD

There are numerous reports of stress negatively impacting reproductive efforts in female animals across multiple species. Yet, the physiological mechanism behind this is still unknown, and the predictability is equally elusive. Previous research from our lab suggests that the reproductive stage of Rainbow Trout (Oncorhynchus mykiss) impacts the sensitivity of the animal to stress. This is measured by the synthesis of the stress hormone, cortisol which is secreted in response to the pituitary adrenocorticotrophic hormone (ACTH). Furthermore, 7α, 20β-dihydroxy-4-pregnen-3-one (DHP), a progestogen reproductive hormone that regulates late-stage ovarian development, increased the in vitro production of cortisol from ACTH-stimulated head kidney tissues. However, the way in which DHP affects cortisol is unknown. Two possible routes are hypothesized. The first is that the levels of ACTH-responsive glucocorticoid receptors, melanocortin 2 receptor (MC2R), are increased leading to a greater sensitivity to cortisol. The second is that cortisol synthesis is enhanced due to increased levels of proteins and/or enzymes. The molecules investigated include cholesterol side chain cleavage enzyme (P450scc), 11β hydroxylase, and steroidal acute regulatory protein (StAR). The use of the in vitro excised head kidney allowed for the direct effects of these hormones to be analyzed without the potential input of the central nervous system. Tissues were treated with ACTH along with a dose response treatment of DHP. Elucidation of the mechanisms connecting stress and reproduction in fish will likely be applicable to all vertebrates, and can help lead to novel insights into preventing stress-related reproductive disorders.

BMS8: Serum 25-Hydroxyvitamin D Concentration is Inversely Associated with Mucosal Inflammation in Patients with Ulcerative Colitis

Austin Kaboff, BA; Joel Pekow, MD; Marc Bissonnette, MD; Katherine Meckel, BA; Stephen Hanauer, MD; Russell Cohen, MD; John Kwon, MD, PhD; David Rubin, MD; Ira Hanan, MD; Atushi Sakuraba, MD, PhD

Vitamin D exerts anti-inflammatory actions both in vitro and in murine models of colitis. In previous studies, we demonstrated that vitamin D protects against the development of colitis by maintaining the integrity of the intestinal mucosal barrier. We sought to evaluate whether deficient serum 25 hydroxyvitamin D [25(OH)D] concentrations are associated with increased mucosal inflammation, a loss of epithelial junctional proteins, and an increase in mucosal inflammatory cytokines in patients with ulcerative colitis (UC). We prospectively enrolled 230 subjects with UC. Serum 25(OH)D concentrations were compared with the Mayo endoscopic score, the total Mayo score, and histologic activity. Colonic mucosal expression concentrations of vitamin D receptor (VDR), E-cadherin, zonula occluden 1 (ZO-1), occludin, claudin-2, tumor

necrosis factor α (TNF-α), and interleukin 8 (IL-8) were compared between dichotomous groups with low or high serum 25(OH)D concentrations. In UC patients, serum 25(OH)D concentration is inversely correlated with mucosal inflammation and disease activity. These results, coupled with the findings that serum 25(OH)D concentrations correlate with the mucosal expression of VDR as well as epithelial junction proteins and inversely with proinflammatory cytokines, suggest that vitamin D deficiency may contribute to UC inflammation by disrupting epithelial barrier function.

BMS9: Human Behaviors Mediate Spread of Lyme Disease Pathogen, Borrelia burgdorferi, by Increasing Rodent Reservoir Host Activities

Matthew McAuliffe, DO Candidate

Human behaviors can have a profound influence on the ecology of an environment. It is proposed that increased human behaviors in peri-urban built environments such as college campuses pose a greater risk for humans to contract Borrelia burgdorferi, the causative agent of Lyme disease. Certain behaviors proliferate risk of transmission to humans by mediating reservoir prevalence, which can in turn influence vector prevalence and vector infectivity rates. We collected ticks by dragging a corduroy cloth over low lying vegetation to attract questing ticks Reservoir species were collected by setting traps baited with peanut butter and oatmeal along walkways and in randomized control grids. Blood and several organs from these reservoir species were then tested for the presence of the Lyme disease pathogen. 2051 blacklegged ticks (I. scapularis) were collected along with 192 competent reservoir species (Peromyscus leucopus and Microtus pennsylvaticus) between July 2013 and November 2014. Thus far, we have determined B. burgdorferi prevalence rates to be over 37% in ticks, and over 50% in the reservoir host, the white-footed mouse. Testing continues to demonstrate that infectivity rates are higher in built environments with high human activity than in wilderness natural environments with low human activity. Based on these results, we believe that increased human behaviors in built environments pose a greater risk to humans for contracting the Lyme disease pathogen by increasing the density of infected ticks and competent reservoir species. These data support the notion of increased risk to humans in high human activity built environments.

BMS10: Potential Treatment of Iron Overloading: Reduced Ttc7 Expression Leads to Robust Urinary Iron Excretion

Alexander Nail, MS; Curtis Rexroth, MS; Amber Wiggins-McDaniel, BS; Robert White, MS, PhD

Iron overload is a serious disease in patients with genetic and transfusion-dependent iron overload. Hereditary Hemochromatosis (HH) results in severe iron overload and significant

morbidity and mortality. Iron deposition occurs in kidney, liver, pancreas and heart which manifests itself in end-organ disease. Treatment of iron overload in HH is by repeated phlebotomy. Patient compliance can be difficult. Iron chelating drugs are also used to decrease iron overload but drug side effects lead to challenges with patient compliance. Patients with transfusion-dependent disease such as beta-thalassemia and sickle cell disease are also non-compliant. A potential alternative treatment for iron overload has been revealed by the study of a mouse mutant exhibiting excessive urinary iron excretion. The fsn mutant mice excretes 100X more iron in urine than normal mice. The mutation in fsn has been identified by our lab in the Ttc7 gene (tetratricopeptide repeat domain 7). Manipulation of TTC7 in humans may induce excretion of iron in urine and represent an effective treatment for iron overload. The goal of this project was to assess the impact of loss of TTC7 function in Hereditary Hemochromatosis to prevent iron overloading. A mouse model of HH, with and without the fsn gene mutation, was generated to determine if iron overloading is prevented by urinary iron excretion. Results of a tissue iron assay showed a statistically significant decrease in iron levels from 1,774 +/- 361 ug/g in HH mice compared to 658 +/- 236 ug/g in HH mice heterozygosis for the fsn gene mutation.

BMS11: A New Mechanism of Action of Dipeptidyl Peptidase-4 (DPP-4) Inhibition on Prevention of Western Diet Induced Aortic Stiffness in female C57-BL6/J mice

Brett Niles, BS; Annayya Aroor, MD, PhD; Guanghon Jia, PhD; Mona Garro, MS; Camila Manrique, MD; Gerald Meninger, PhD; Thomas Klein, PhD; James Sowers, MD; Vincent DeMarco, PhD; Javad Habibi, PhD

We recently reported that Western diet(WD) high in fat and refined sugars induces aortic stiffness and endothelial dysfunction in female C57-BL6/J mice mice. There has been much interest lately in the potential vaso-protective properties of DPP-4 inhibitors. Interestingly, FGF-23/Klotho is increasingly recognized in the regulation of vascular stiffness. The purpose of this study was to determine whether DPP-4 inhibition prevents WD-induced vascular stiffness and whether this efficacy is mediated in part by FGF23/Klotho signaling in the vasculature. Four week old mice were fed control diet(CD) or WD with or without the DPP-4 inhibitor, linagliptin (LINA) for 16 weeks, after which aortic stiffness, endothelial stiffness and aortic vascular reactivity were examined by vascular ultrasound (pulse wave velocity-PWV), atomic force microscopy and wire myography, respectively. Compared to CD mice, WD mice exhibited 21% and 353% higher aortic PWV and endothelial stiffness, respectively and these vascular abnormalities were prevented by LINA. LINA also prevented WD-induced impairments in acetylcholine, sodium nitroprusside, and insulin-mediated aortic vascular relaxation. Analysis of bright-field images showed that the significant increases in peri- aortic fibrosis, medial thickness and 3-nitrotyrosine observed in WD- fed mice were prevented by LINA. Likewise, immunofluorescence analysis revealed that consumption of a WD resulted in higher expression level of FGF-23, osteopontin and lower expressions of klotho and PTHrP and LINA prevented

these abnormalities. In summary, these findings support the notion that DPP-4, LINA prevents the development of vascular stiffness, remodeling and dysfunction and that this protection may involve modulation of FGF-23, klotho, and PTHrP in aorta.

BMS12: The Production, Purification, and Post-Translational Modification of PvdJ Module 2 in Pseudomonas Aeruginosa

Dharam Patel, BS; Kathleen Meenely, PhD; Annemarie Chilton, BS; Audrey Lamb, PhD

A growing problem in modern medicine is antibiotic resistance in bacterial species that cause life threatening infections. One such example is the opportunistic pathogen Pseudomonas aeruginosa, a leading cause of lung infections among cystic fibrosis patients. An area of study for P. aeruginosa is siderophore production. In order for P. aeruginosa to grow and survive in the host environment iron uptake is important. One method this pathogen uses is the production of siderophores, which are low molecular iron chelators comprised primarily of amino acids that assist in uptake of iron from the host environment. P. aeruginosa uses non-ribosomal peptide synthetase (NRPS) enzymes which act as the assembly line for siderophore production. For this project the second module of PvdJ (PvdJ M2) protein, which is used in the production of the siderophore pyoverdin, will be analyzed. The specific amino acid that this module incorporates into the siderophore is formyl-hydroxyornithine. PvdJ M2 was overproduced in BL21(DE3) Escherichia coli at 15 °C for 21 hours and protein production was induced with IPTG. The cells were lysed using the French Press and protein purification was carried out using fast protein liquid chromatography. The protein was then post translationally modified in vitro using a phosphopantethienyl transferase (PPTase) and coenzyme A. Confirmation of the activation by post translational modification was attempted by the Ellman's assay. Future work includes development of a fluorescent assay for confirmation of post-translational modification, the determination of kinetic constants through an adenylation assay, and crystallization to determine the three dimensional structure.

BMS13: Coordinate Action of Distinct Sequence Elements Localizes Checkpoint Kinase Hsl1 to The Septin Collar at the Bud Neck in Saccharomyces cerevisiae

Aspram Sargsyan, BA; Jeremy Thorner, PhD; Gregory Finnagan, PhD; Angela Duvalyan, Student; Elizabeth Liao, Student

Passage through the eukaryotic cell cycle requires processes that are tightly regulated both spatially and temporally. Surveillance mechanisms (checkpoints) exert quality control and impose order on the timing and organization of downstream events by impeding cell cycle progression until the necessary components are available, undamaged and have acted in the proper sequence. In budding yeast, a checkpoint exists that does not allow timely execution of the G2/M transition unless and until a collar of septin filaments has properly assembled at the

bud neck, which is the site where subsequent cytokinesis will occur. An essential component of this checkpoint is the large (1518-residue) protein kinase Hsl1, which only localizes to the bud neck if the septin collar has been correctly formed. Hsl1 reportedly interacts with particular septins; however, the precise molecular determinants in Hsl1 responsible for its recruitment to this cellular location during G2 have not been elucidated. We performed a comprehensive mutational dissection and accompanying image analysis to identify the sequence elements within Hsl1 responsible for its localization to the septins at the bud neck. Unexpectedly, we found that this targeting is multipartite. Hsl1 construct with residues 611-950 and 1245-1518 was found to be necessary and sufficient for budneck recruitment both in vitro and in vivo.

BMS14: Identification of Human FANCD2 Structural Domain(s) Integral to DNA Repair via Interaction with Bloom Helicase

Nikitha Sathish Kumar, BS; Alexandra Sobeck, PhD; Maya Raghunandan, Post Doctoral Student

Fanconi Anemia (FA) and Bloom Syndrome (BS) are closely related inheritable genomic instability diseases, characterized by a cellular inability to repair DNA damage. FA is caused by mutations in any one of 16 FA genes that encode proteins participating in the FA DNA repair pathway. BS is caused by mutations in one single gene, BLM. One FA disease variant of particular interest in this study results from a mutated Fanconi Anemia D2 (FANCD2) gene. FANCD2 encodes for a central protein in the FA DNA repair pathway. Likewise, mutated BLM encodes Bloom Helicase (BLM), a key protein in the BLM pathway. Crosstalk between FA and BLM pathways is partially mediated through interaction of BLM with FANCD2; moreover colocalization of FANCD2 and BLM has been observed in response to DNA replication arrest. This study aimed to map the site of FANCD2-BLM interaction on FANCD2 in a human cellular system. In 2011, the crystal structure of FANCD2 was solved and revealed that FANCD2 is comprised of 4 solenoid (S1, S2, S3, S4) and 2 helical bundle (HD1, HD2) structural domains. Our knowledge of these structural domains in FANCD2 aided in the creation of several truncated human FANCD2 (hFANCD2) mutants. These mutants were then going to be tested for their ability to interact with human wild type BLM.

BMS15: SOX9 is Targeted for Proteasomal Degradation by the E3 Ligase FBW7 in Response to DNA Damage

Jamie Shah, BA; Xuehui Hong, MD, PhD; Wenyu Liu, PhD; Ruipeng Song, PhD, Xing Feng, BS; Chi-Kwan Tsang, PhD; Katherine Morgan, BS; Samuel Bunting, PhD; Hiroyuki Inuzuka, PhD; Sharon Pine, PhD

SOX9 encodes a transcription factor that governs cell fate specification throughout development and tissue homeostasis. Elevated SOX9 is implicated in the genesis or progression

of human tumors though increasing cell proliferation and epithelial-mesenchymal transition. We found that in response to UV irradiation or genotoxic chemo-therapeutics, SOX9 is actively degraded by a ubiquitin pathway-dependent mechanism across different tumor types including lung cancer, colon cancer, osteosarcoma, and melanoma, as well as normal human bronchial epithelial cells. We found that SOX9 is phosphorylated by GSK3β at Ser-236, facilitating the direct binding and degradation of SOX9 via the F box protein, FBW7α. We also determined that the de-ubiquitinase, USP28, stabilizes SOX9 under normal conditions by sequestering FBW7, but releases FBW7 after UV irradiation, allowing FBW7 to bind SOX9 and target it for destruction. DNA damage-induced SOX9 degradation was independent of p53, ATM, ATR and DNA-PK. Failure to deplete SOX9 attenuated the DNA damage-induced intra-S-phase checkpoint and increased long-term cell survival. Moreover, mutations within the FBW7 phosphodegron binding site of SOX9 prevented SOX9 degradation after DNA damage, and incurred resistance of non-small cell lung cancer (NSCLC) cells to cisplatin in vivo. Patients with tumors expressing high SOX9 and low FBW7 levels exhibit inferior survival. Our discovery reveals a novel function of SOX9 in the cellular response to DNA damage. Induced degradation of SOX9 may be part of the protection mechanisms to maintain genomic stability. This new regulatory mechanism of the FBW7-SOX9 axis in cancer could have diagnostic and therapeutic implications.

BMS16: Interferon-γ Inhibits Vaccinia Virus Replication and Spread in Lung Epithelial Cells by Inhibiting Actin Polymerization

Kyle Varkoly, BS; Sharahm Salek-Ardakani, PhD; Vikas Tahiliani, PhD; Tarun Hutchinson, PhD

Several orthopox viruses cause clinical diseases in humans, such as variola virus (VARV), monkeypox, cowpox, and vaccinia virus (VACV). VARV, the etiological agent of human smallpox, is highly infectious by aerosol transmission in a susceptible population. Using a highly virulent respiratory VACV infection model to simulate the natural route of human smallpox and monkeypox infection, we found that large numbers of activated and functionally proficient CD8+ T cells are rapidly recruited to the lung. These are found in close proximity to virus-infected bronchial lung epithelial cells and release interferon γ (IFN-γ) to inhibit VACV replication and dissemination to visceral tissues. To enhance virus spread, vaccinia virus has evolved complex strategies to direct the formation of actin tails propelling them toward neighboring cells. Investigating the precise molecular mechanisms of action of IFN-γ, we discovered that interferon γ attenuates VACV-induced actin polymerization by inhibiting the Wiskott-Aldrich syndrome protein (NWASP) and ARP2/3 (Actin Related Protein) interaction and nucleation of actin filaments in mouse and human lung epithelial cells. Our observations uncover an unexpected role for IFN-γ during virus spread and have important implications for discovery of future vaccines and therapeutics for existing and emerging human respiratory virus infections.

BMS17: Neuroendocrine Disruption by 17α-Ethinylestradiol and Bisphenol-A in Zebrafish

Taelah Wooten, MS

Aquatic organisms are highly susceptible to endocrine disruption due to increased accumulation of estrogenic endocrine disrupting chemicals (EDCs) in surface waters. The consequence of EDC exposure has not been well characterized in the adult male reproductive neuroendocrine system, although there is considerable circumstantial evidence for neuroendocrine disruption based on limited information available in the literature. This study was designed to evaluate the effects of environmental contaminants 17α-ethinylestradiol (EE2) and bisphenol-A (BPA) on adult male zebrafish reproduction. It was hypothesized that EE2 and BPA will act as estrogen mimics to inhibit the male hypothalamus-pituitary-gonadal (HPG) axis. In the first experiment, adult male zebrafish in three treatment groups were injected with 0.1, 1, or 10 nmol/kg EE2, once a week for three weeks. At the end of the three weeks, whole brain tissue samples were collected and analyzed for changes in Kisspeptin (Kiss) content, gonadal samples were collected for evaluation of any histopathological changes and water samples were collected to evaluate sex steroid production. A similar experiment was performed using 0.1,1, and 10 µmol/kg BPA injections and the same neuroendocrine and reproductive parameters were evaluated after the 3-week exposure period. EE2 (10 nmol/kg) injections significantly increased Kiss content, decreased the size of the testes and inhibited production of sperm. BPA exposure at the doses used in the present study induced non-significant alterations in the measured parameters. Results of this study show that EE2 and BPA, can act at multiple levels of the HPG axis to disrupt male reproductive system.

Clinical Sciences - Students

CS1: A Mouse Model o Assess Neointimal Formation Following Endothelial Denudation and Bioresorbable Stent Implantation

Ashkon Nehzati, Biomedical Engineering; Dariush Davalian, Abbott Vascular; Jesus Magana, Abbott Vascular; Paul Consigny, PhD; Trevor Cardinal, PhD

Restenosis is a common response to bare-metal stent implantation occurring in 16-44% of patients. Drug-eluting stents can reduce restenosis, but late-stent thrombosis occurs in 0.4-0.6% of patients following cessation of anti-platelet therapy. Bioresorbable stents may limit both restenosis and late thrombosis. The goal of this work is to develop a mouse model that can be used to assess neointimal formation in response to injury and/or bioresorbable stent implantation. The endothelium was denuded in the abdominal aorta of C57Bl/6 mice using a nitinol device inserted into the femoral artery and confirmed using Evans Blue staining and histopathology. Bioresorbable stent implantation was performed by placing a stent (polymeric coil) in a PE tube, and advancing it into the abdominal aorta. Using a wire advancer, the stent was placed while withdrawing the tube simultaneously. Staining and histopathology revealed endothelial denudation of the abdominal aorta between the renal arteries and iliac bifurcation during acute procedures and increases in wall thickness and cellularity over 28 days. Direct visualization revealed that the polymeric stent can be delivered to area of endothelial denudation with no migration or distal embolization. In summary, we created a mouse model to assess neointimal formation following endothelial denudation and/or bioresorbable stent deployment. In the future, this model can be used to characterize gene expression and assess specific molecular pathways that contribute to restenosis and thrombosis.

CS2: Effect of Probiotics on the Acute Stress Response

Aarthi Ramesh, MS; David Fraser, PhD

Background: There is now compelling evidence for a link between the GI microbiome, brain function, and behavior. Multiple studies have shown that ingestion of probiotics modulates the processing of information that is linked to anxiety, depression, and influences the neuroendocrine stress response. This study explored the effects of Lactobacillus Rhamnosus GG on the secretion of cortisol in healthy volunteers. Methods: 20 masters-level students received either daily probiotic supplements or a placebo for 4 weeks. Salivary cortisol samples were collected before and after completion of a MAAST Stress Test, during two separate sessions (pre and post intervention). After completion of the stress test, participants completed a computerized battery of cognitive tests assessing performance, which were specifically related to working memory and attentional biases related to the processing of emotionally salient information. Results: After 4-Weeks of probiotic supplementation, the Experimental Group (i.e.

Probiotic Intervention Group) demonstrated greater habituation of the HPA axis upon re-exposure to an acute stressor (MAAST Stress Test) than the Control Group. However, the sample size was not large enough to prove statistical significance. Discussion: The suppression of the neuroendocrine stress response and the increase in the processing of positive versus negative attentional vigilance in subjects supplemented with probiotics are consistent with previous findings of endocrine and anxiolytic effects of a different lactobacillus rhamnosus strain. Further studies are therefore needed to test the utility of LB supplementation in the treatment of stress-related disorders, specifically in clinical populations with depression and anxiety disorders.

Clinical Sciences - Resident Faculty

CS3: Self-Administered Pneumatic Insufflation for the Treatment of Subjective Tinnitus.

Caleb Masterson, MS; Larry McIntire, DO

Objectives: This study was conducted to determine the effectiveness of self-administered pneumatic insufflation of the tympanic membrane in the palliation of subjective tinnitus. Tinnitus is a phantom auditory perception that occurs in humans. Methods: We conducted a retrospective chart review in a community otologist office, of patients complaining of tinnitus successfully treated with pneumatic insufflation of the tympanic membrane (TM). Pneumatic insufflation was demonstrated to the patients using an infant bulb irrigator and a rubber otoscopic tip. Patients included were without other otological pathology whom confirmed treatment effectiveness with office insufflation. There were 26 patients that met criteria. Patients data including initial Disease-Specific Symptom Score (VAS) grading of tinnitus, post home treatment VAS, audiogram and duration of symptoms were catalogued and analyzed. Results: Significant reductions in subjective symptoms were noted among in patients using continued at home insufflation compared to control ($P < .0001$ $n = 15$; $P < .234$ $n = 9$, respectively). Additionally, patients with symptoms less than 1 week also experienced significant reductions in symptoms ($P <.0001$, $n= 8$) as compared to those with symptoms exceeding 1 weeks ($P < .005$, $n=17$), both significant to control. Conclusions: Taken together, our results provide evidence that self-administered pneumatic insufflation of the TM patients with subjective tinnitus can reduce symptoms. Further investigations are warranted and would include a measurement in the duration of reduction of symptoms.

Clinical Sciences - Residents

CS4: Medical Clearance of the Clinically Medically Stable Psychiatric Patients in the Emergency Department

Jordan Birch, DO; Samantha Egbert, DO; Frank Veer, DO

Almost every emergency department is facing an ever increasing number of patients presenting with psychiatric complaints. For many patients requiring inpatient psychiatric treatment, emergency departments are required to provide medical screening exams. No universal guidelines have been put in place for such exams, but many states and facilities require specific testing and laboratory work for a patient's final disposition. While many patients require further testing, there is still a subset of stable psychiatric patients with normal vitals, unaltered cognition and no signs of delirium that still receive mandated testing. This study was a retrospective cohort study looking at efficacy of mandatory testing in the medically stable patients and to see if these tests incurred any change in patient's disposition from the emergency department. 490 consecutive presentations of patients presenting for psychiatric complaints at a 40 bed community based hospital emergency department were reviewed. Chief complaint, vital signs, history pertaining to the complaint, history of substance abuse as well as physical exam findings, lab results and disposition of the patient were reviewed. We excluded patients less than 18 years of age, medically unstable, lethargic, unresponsive, pertinent medical comorbidities, major trauma, physical exam findings that required emergent treatment, current medication or illicit drug overdoses. Multiple abnormal labs were found, mostly ranging from hypokalemia, anemia and urinary tract infections, with a small subset receiving treatment. Our review showed that mandatory labs did not review any subsequent pathology with no re-admission for further medical treatment or alteration in their treatment at a psychiatric facility.

CS5: Predictive Values of Parathyroid Surgery in a Community Hospital in Southern Missouri

Sheppard Peng, DO; Scott McClintick, DO

Parathyroid surgery for primary hyperparathyroidism (PHPT) has evolved tremendously over the last 30 years. The trend is been moving away from comprehensive bilateral neck exploration and moving towards a minimally invasive, localization guided parathyroidectomy. The advantages of this include smaller scar, shorter operative time, fever complications, while maintaining similar cure rates. The minimally invasive approach relies on preoperative and intraoperative findings that assist the surgeon in decision making. The most important adjunct developed is the intraoperative parathyroid hormone (IOPTH) testing. Classic criteria for success using this test are at least a 50% drop in PTH levels compared with preoperative values. At our institution, this test often takes >30 minutes, which prolongs the operating time,

especially if the level does not fall. Surgeons could perform a bilateral exploration during the time it takes to get the IOPTH level back and this delay leads to frustration. Operating room time is expensive and increased time of anesthesia carries additional risks to the patient. The aim of this study is to identify preoperative and intraoperative factors that predict the likelihood of additional hyperfunctioning glands and guide the surgeon in deciding whether to wait for the IOPTH or to continue exploring further.

CS6: Re-Treatmentent of Generalized Granuloma Annulare with Adalimumab

Michael Zaycocksy, MS, DO; Jeffrey Collins, DO; Jere Mammio, DO

Granuloma annulare is a fairly common dermatologic condition caused by dermal and subcutaneous inflammation. It has a female to male predilection of 2:1 and can be aesthetically displeasing to the patient depending on the location of the lesions. While the condition is benign and self-limiting, it can take several years for complete clearance. Generalized granuloma annulare occurs in a small portion of cases and is particularly treatment resistant.This case report discusses a 45 year old female with generalized granuloma annulare who trialed topical steroids, dapsone, pentoxifylline, and isotretinoin with minimal improvement. She was then treated with adalimumab and rapidly cleared. This was tapered completely and she remained clear for 7 months. Adalimumab was restarted and she cleared rapidly once again. When dealing with treatment resistant generalized granuloma annulare, adalimumab may be a welcome addition to the numerous treatment options.

Medical Education - Faculty

ME1: Show & Tell - Some of My Online Worksheets and Ideas for The Future

Dennis Wolff, PhD;

I have generated a number of worksheets, etc., over the years that were used to facilitate student learning. The ideas I have employed or envision have the potential to assist students in multiple areas of study where terminology must be learned, other memorization is required, opportunities to practice calculations are needed, etc. Examples of these which will be demonstrated include: - Crossword Puzzles: Used for vocabulary (e.g., for renal physiology) and pharmacology content. Multiple versions of same puzzles are made with 1/4 letters free. Students can't ask for the answer to 1D but can work together by discussing clues. Work can be saved and submitted/graded online. - Dynamic Worksheets: Random number generators are used to generate worksheets in which the same methods are used to obtain different correct answers. Used for pharmacokinetics, clearance and acid-base calculations. Progress can be saved and submitted work is automatically graded. - Excel Worksheets: Excel graphic capabilities were used to show the impact of changes in drug dose, rate of absorption, rate of elimination, pharmacogenetics, etc., on circulating drug levels. Another uses macros to generate a dose-response curve the placed with others on a graph to assess rank orders of potency and efficacy. Ideas for a new game, student-written question sharing platform, and a pharmacology flashcard database will be discussed among others. Students can help make some of these things a reality. Also solicited will be ideas for optimum utilization as some students resent "homework" assignments and refuse to complete if optional regardless of benefits.

Medical Education - Students

ME2: Comparison of Umbilical Cord Occlusion Methods: Radiofrequency Ablation Versus Laser Photocoagulation

Mira Abdel-Sattar, BS; Andrew Chon, MD; Arlyn Llanes, RN; Lisa Korst, MD, PhD; Joseph Ouzounian, MD; Ramen Chmait, MD

OBJECTIVE: To compare outcomes between two umbilical cord occlusion (UCO) methods: radiofrequency ablation (RFA) versus fetoscopic-guided laser photocoagulation. METHODS: A retrospective study of prospectively collected data was performed on all monochorionic-diamniotic multiple gestations that underwent UCO with RFA or laser between May 2006 and May 2016. During the ten-year study span, there was a shift from laser to RFA due to operator preference and equipment availability. In bivariate analysis, patients treated with RFA were compared to those with laser. Risk factors for intrauterine fetal demise (IUFD) and 30-day

survival of the co-twin were identified, and multiple logistic models were fitted to examine whether the UCO method was associated with these outcomes. RESULTS: Of 60 UCO cases, 18 (30%) underwent RFA and 42 (70%) underwent laser surgery. The RFA method was associated with co-twin IUFD after surgery (6/18 [33.3%] vs 1/42 [2.4%], P = 0.0021). In logistic regression models, patients who underwent RFA were more likely than patients who underwent laser to have an IUFD of the co-twin (OR 13.2, 1.23-142.62, P = 0.0331). These patients were also less likely to have 30-day survival of the co-twin, although this was not statistically significant (OR 0.20, 0.04-1.15, P = 0.0710). CONCLUSION: Despite clear technical advantages of RFA compared with laser, including ease of surgical access and decreased invasiveness, RFA appeared to be associated with increased risk of post-procedure fetal demise. Before recommendations can be made regarding preferred UCO treatment modality, further study is warranted.

Medical Education - Residents

ME3: A Comparison of Three Methods For Ossicular Chain Reconstruction

Andrew Van Bruggen, DO; Larry McIntire, DO; Kindall Martin, ATSU-COM

Ossicular chain reconstruction is frequently employed to restore continuity of the ossicular chain and thus reduce conductive hearing loss. Dysruption of the middle ear bones may result from loss of adequate mucosal vascular supply, infection or trauma. The hearing outcome of reconstruction is to a great extent dependent upon the defect being corrected. Building from an intact stapes superstructure enhances the likelihood of hearing restoration. Total ossicular chain reconstruction via a columellar strut from the stapes footplate to the tympanic membrane often results in continued conductive hearing loss. Because of the importance of stable, readily available as well as cost effective reconstruction, we conducted a study that compares and contrasts three different grafting materials. Harvested cortical bone grafts were compared to manufactured and packaged prostheses and tragal cartilage in reconstruction of the middle ear hearing mechanism. A literature review was performed as well as chart reviews of OCR on a small population of our patients. These results were comprehensively studied and reviewed to provide a confident, evidence based, recommendation on the topic discussed.

ME4: A case of Multiple Sclerosis Presenting as Wernicke's Encephalopathy

Brandon Vanhessche, DO;

Background Multiple sclerosis (MS) has a prevalence of approximately 149 per 100,000 individuals in the US. Patients may present as an acute or chronic exacerbation of a psychiatric or neurologic disorder such as depression. Standard treatment is high dose steroids, but supporting documentation exists of improvement utilizing high dose thiamine. (Frederick 1973)

Methods We present a case of a patient receiving high dose IV thiamine for suspected Wernicke's encephalopathy (WE) that was subsequently diagnosed with MS. We will review treatment response and support for continued study. Results A 30-year-old female was admitted for severe PTSD and Depression after a multi-month decline and 100lb weight loss. Presentation and decline led to suspicion of WE in a malnourished patient. Parenteral thiamine provided symptomatic improvement in the patient while awaiting brain imaging studies. MRI showed lesions compatible with WE, but also suggested MS. Confirmatory CSF findings established MS. Additional steroid administration continued improvement of symptoms. Discussion There has been a dearth of research and documented of use of thiamine in MS. Thiamine presents a unique treatment strategy due to minimal side effects, and in small studies shows significant improvement of fatigue contributing to overall improvement. (Constantini 2013) This case also demonstrates the difficulty of identifying MS in a psychiatric setting as symptoms have significant crossover with WE and depression. Conclusion Thiamine's role as a treatment modality for MS should not be overlooked due to its high propensity for improvement of fatigue, mood, and outlook, and lack of significant associated side effects.